Gen5
Sample Protocols & Experiments Guide

BioTek®

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Overview

Numerous sample protocols are shipped with Gen5. You can use the protocols to learn more about Gen5 and as a timesaver, customizing them to meet your needs and then running them in an experiment to obtain results.

> ❖ **Recommendation**: Before making any modifications to the sample protocols, open them and select **File>Save As** to assign a unique name to the protocol.This will preserve the original sample protocol for future use.

A matching experiment file is also shipped with Gen5 for use as a learning tool. Many of the experiment files contain actual data so you can see how Gen5 presents the results on-screen and in reports.

Find the sample protocols and experiments shipped with Gen5 in the default file storage locations. A folder for each detection method is provided: **Absorbance**, **Fluorescence**, **Luminescence**, and for Synergy 2 users, there is a **Synergy 2** folder within each detection method folder.

Sample Files Location:

Gen5 Secure (and database users): Select **File>Open Protocol**, in the DB directory select the Samples folder.

All other levels of Gen5: Select **File>Open Protocol** and browse to **C:/Program Files/BioTek/Gen5/Samples**

> ❖ **Important**: The sample protocols must be considered as examples provided for demonstration and guidance purposes. If you plan to use these protocols or similar ones in a real application, it is your responsibility to validate the protocol parameters, including the report and export content (if applicable), before using them.
>
> ❖ **Notes**: Your system administrator can change the path and filenames described above. If you cannot find the Samples folder, contact your system administrator. Also note, your reader may not support all of the sample protocols provided. Review the descriptions in this guide to see if your reader is compatible with the defined steps.

Absorbance

This section provides descriptions of the absorbance protocol and experiment files shipped with Gen5.

260/280 Ratio Test

Basis for the Assay:

The presence of protein impurities inhibits many experimental reactions that are performed on nucleic acids. Therefore it is paramount that an assessment of the level of protein contamination of nucleic acid samples be performed prior to any experiments.

Protocol name: 260_280 Ratio.prt

The protocol calls for an absorbance measurement at 260 nm and 280 nm. Background absorbance of the microplate is removed by subtraction of a blank well. Using a Transformation the A_{260}/A_{280} ratio is calculated. *Wells with a ratio value of less than 1.7 are considered to be positive for the presence of protein.* This is expressed in a Cutoff data set.

Experiment File Name: 260_280 Ratio Test.xpt

The experiment data file does not contain any data

Plate Configuration:

The plate layout places one blank in position A1. The rest of the wells contain 95 individual samples

Report:

The Report is configured to provide the (1) blanked 260 nm absorbance; (2) blanked 280 nm absorbance and (3) Calculated 260/280 ratio. A Plate report using a cutoff of 1.7 on the 260/280 ratio indicating the possible presence (POS) or absence (NEG) of protein is also reported.

AlamarBlue™ (Absorbance)

Basis for the Assay:

The internal environment of proliferating cells is more reduced than that of non-proliferating cells. Compounds such as tetrazolium salts and alamarBlue™, which can be reduced by cellular metabolic intermediates, can be used to monitor cellular proliferation. The reduction of AlamarBlue results in a measurable color shift. The wavelengths for maximal absorbance are 570 nm and 600 nm for the reduced and oxidized forms of alamarBlue respectively. Because the wavelengths overlap it is necessary to measure the absorbance at both wavelengths.

Protocol name: AlamarBlue-A.prt (Absorbance Readers)

The protocol calls for absorbance measurements at **570 nm** and at **600 nm**.

Experiment File Name : NA

Plate Configuration:

Plate configuration provides for samples to be run in duplicate. In addition, two blank wells (Media only) and two control-wells (media plus alamarBlue) are defined on the plate. In regards to data reduction, a transformation that calculates reduction percentage using the molar extinction coefficients of reduced and oxidized alamarBlue at 570 nm and 600 nm has been created.

Report:

The report is configured to provide the (1) Blanked absorbance at 570 nm and 600 nmf and (2) Percent reduction calculation.

BCA Protein Assay

Basis for the Assay:

Quantitation of total protein content is a measurement common to many applications in basic science and clinical research. The basis for this assay is the reduction of copper in alkali by protein (biuret reaction) in the presence of the chromogenic agent bicinchoninic acid. This reaction produces a blue color with an absorbance peak at 562 nm.

Protocol name: BCA Protein Assay.prt

The protocol calls for an endpoint absorbance measurement at **562 nm**. Plate map with a standard curve from 0 to 100 µg/ml with samples and standards in duplicate. Linear regression curve fit is used to determine unknown concentrations.

Experiment file name: BCA Protein Assay with data.xpt

The experiment file contains the absorbance determination. Transformed (blank-subtracted) data is used to plot the standard curve.

Plate Configuration:

The plate layout includes standards in duplicate, with concentrations of 0 to 100 µg/ml placed in wells A2 through B6. Reagent blanks are in A1 and B1. 42 unknown samples are entered in duplicate on the layout.

Report:

The standard curve is reported along with equation parameters and correlation coefficients. A matrix report is configured to provide the (1) 562 nm absorbance and (2) calculated protein concentrations. A column type report contains the statistics for standards and samples.

BioCell Layout Protocol

Basis for the Assay:

The patented 1 cm quartz BioCell can be read in any of BioTek's automated microplate readers to allow quick readings of sample sizes with results comparable to standard cuvette measurements.

Protocol Name: BioCell Layout.prt

The protocol shows 1 read step at a **450 nm** wavelength. The wavelength can be easily modified for the required wavelength(s)

Experiment File Name: BioCell Experiment.xpt

This experiment uses the BioCell Layout.prt and has one plate of data (containing data from reads of 8 individual BioCells).

Plate Configuration:

The plate layout indicates samples 1 through 8 corresponding to the 8 locations for BioCells in the BioCell adapter plate. The plate layout can be modified to show only those locations that will actually be used.

Report:

The BioCell reports OD for up to 8 BioCells having been read in the microplate reader.

Bradford Protein Assay

Basis for the Assay:

Quantitation of total protein content is a measurement common to many applications in basic science and clinical research. The basis for this assay is the binding of Coomassie Brilliant Blue G-250 to protein with a resultant shift in the absorbance maximum from 465 to 595. Absorbance at 595 nm is used to quantitate protein content.

Protocol name: Bradford Protein Assay.prt

The protocol calls for an endpoint absorbance measurement at **595 nm**. Plate map with a standard curve from 0 to 100 µg/ml with samples and standards in duplicate. Linear regression curve fit is used to determine unknown concentrations

Experiment file name: Bradford Protein Assay with data.xpt

The experiment file contains the absorbance determination, which is used to plot the standard curve.

Plate Configuration:

The plate layout includes standards in duplicate, from 0 to 100 µg/ml, in well positions A1 through B5. Samples are also in duplicate, for a total of 43 samples in this example.

Report:

The report includes the standard curve, and it's equation parameters and correlation coefficients. A matrix report is configured to provide the (1) 595 nm absorbance and (2) calculated protein concentrations. A column report contains the statistics for standard and sample concentrations.

Direct dsDNA Quantitation

Basis for the Assay:

The aromatic ring structure of the purine and pyrimidine moieties that make up the nucleoside bases of DNA and RNA are responsible for absorbance of UV light at 260 nm. Although each specific base has a maximal absorbance at a slightly different wavelength, on average, nucleic acids as a macromolecule will absorb maximally very near 260 nm. In terms of quantitation, different nucleic acid species have different average extinction coefficients. By converting these known extinction coefficients to a specific concentration for a particular absorbance value at a defined wavelength, the nucleic acid concentration of solutions can be determined by a simple absorbance measurement. For example, the extinction coefficient for dsDNA (1 mg/ml) at 260 nm is 20 ODs for a 1 cm pathlength; this can be recalculated to mean 1.0 OD has a concentration of 50 µg/ml.

Protocol name: dsDNA Quantification Blank Plate.prt

The protocol calls for a pre-read of the microplate to subtract the background absorbance of the microplate at 260 nm, followed by an absorbance measurement at **260 nm**. Pathlength correction is selected to correct the absorbance value to reflect a pathlength of 1 cm. The corrected absorbance is then used in a single plate transformation to calculate DNA concentration.

Experiment file name: dsDNA Quantification Blank Plate with data.xpt

The experiment file contains all of the absorbance determinations necessary including the pre-read plate (260 blank), the absorbance determinations at 977 nm (Test) and 900 nm (Reference), which are utilized to correct for pathlength, as well as the 260 nm experimental determination.

Plate Configuration:

Plate configuration includes samples in duplicate. There are no blanks or controls.

Report:

The report contains a matrix with:
1. Raw absorbance values
2. Absorbance corrected to 1 cm pathlength
3. Calculated DNA concentration.

The report also contains a table with the corrected ODs, calculated concentrations and standard deviation and % CV of the replicate sets.

Direct Oligonucleotide Quantitation

Basis for the Assay:

The aromatic ring structure of the purine and pyrimidine moieties that make up the nucleoside bases of DNA and RNA are responsible for absorbance of UV light at 260 nm. Although each specific base has a maximal absorbance at a slightly different wavelength, on average, nucleic acids as a macromolecule will absorb maximally very near 260 nm. In terms of quantitation, different nucleic acid species have different average extinction coefficients. By converting these known extinction coefficients to a specific concentration for a particular absorbance value at a defined wavelength, the nucleic acid concentration of solutions can be determined by a simple absorbance measurement. For example, the extinction coefficient for ssDNA oligonucleotides (1 mg/ml) at 260 nm is 13 ODs for a 1 cm pathlength; this can be recalculated to mean 1.0 OD has a concentration of 32.5 µg/ml.

Protocol name: Direct Oligo Quantification with Blank Plate.prt

The protocol calls for an absorbance measurement at **260 nm** along with a pre-read of the microplate to subtract background absorbance of the microplate. Pathlength correction has also been selected to correct the absorbance value to reflect a pathlength of 1 cm. The corrected absorbance is then used to calculate oligo concentration.

Experiment file name: Direct Oligo Quantification with Blank Plate with data.xpt

The experiment file contains all of the absorbance determinations necessary including the pre-read plate (260 blank), the absorbance determinations at 977 nm and 900 nm, which are utilized to correct for pathlength, as well as the 260 nm experimental determination.

Plate Configuration:

Plate configuration includes samples in duplicate. There are no blanks or controls.

Report:

The report is configured to provide the (1) raw absorbance, (2) pathlength corrected absorbance statistics, and (3) calculated oligonucleotide concentration statistics.

Direct RNA Quantification

Basis for the Assay:

The aromatic ring structure of the purine and pyrimidine moieties that make up the nucleoside bases of DNA and RNA are responsible for absorbance of UV light at 260 nm. Although each specific base has a maximal absorbance at a slightly different wavelength, on average, nucleic acids as a macromolecule will absorb maximally very near 260 nm. In terms of quantitation, different nucleic acid species have different average extinction coefficients. By converting these known extinction coefficients to a specific concentration for a particular absorbance value at a defined wavelength, the nucleic acid concentration of solutions can be determined by a simple absorbance measurement. For example, the extinction coefficient for RNA (1 mg/ml) at 260 nm is 16 ODs for a 1 cm pathlength; this can be recalculated to mean 1.0 OD has a concentration of 40 µg/ml.

Protocol name: Direct RNA Quantification Blank Plate.prt

The protocol calls for an absorbance measurement at **260 nm** along with a pre-read of the microplate to subtract background absorbance of the microplate. Pathlength correction is used to correct the absorbance value to reflect a pathlength of 1 cm. The corrected absorbance is then used to calculate RNA concentration.

Experiment file name: Direct RNA Quantification Blank Plate with data.xpt

The experiment file contains all of the absorbance determinations necessary including the pre-read plate (260 blank), the absorbance determinations at 977 nm (Test) and 900 nm (Reference), which are utilized to correct for pathlength, as well as the 260 nm experimental determination.

Plate Configuration:

Plate layout includes samples in duplicate. There are no blanks or controls.

Report:

The report contains a matrix with:
1. Raw absorbance values
2. Absorbance corrected to 1 cm pathlength
3. Calculated RNA concentration.

The report also contains a table with the corrected ODs, calculated concentrations and standard deviation and % CV of the replicate sets.

EDTA Inhibition Assay

Basis for the Assay:

The presence of EDTA inhibits divalent ion (e.g. Mg^{++}, Mn^{++}) dependent reactions. The A_{260}/A_{230} ratio for pure DNA is approximately 2.0, values less than 2.0 suggest the presence of EDTA contamination.

Protocol name: EDTA Inhibition Assay.prt

The protocol calls for an absorbance measurement at 230 nm and 260 nm. Background absorbance of the microplate is removed by subtraction of a blank well. A transformation is used to calculate the A_{260}/A_{230} ratio. *Wells with a ratio value of less than 2.0 are considered to be positive for the presence of EDTA.*This is expressed in a Cutoff data set.

Experiment File Name: EDTA Inhibition Assay.xpt

The experiment data file does not contain any data.

Plate Configuration:

The plate layout places one blank located in position A1. The rest of the wells contain 95 individual samples.

Report:

Plate Report is configured to provide the (1) blanked 230 nm absorbance; (2) blanked 260 nm absorbance and (3) Calculated 260/230 ratio. A Plate report using a cutoff of 2.0 on the 230/260 ratio indicating the possible presence (POS) or absence (NEG) of EDTA is also reported.

IC50 (Toxicity) Assay

Basis for the Assay:

In a typical competitive binding assay the primary measure of the dose-response curve is the IC50 or the concentration that provides 50% inhibition. In this example, it is first necessary to calculate the theoretical % toxicity of the samples by dividing the mean blanked sample ODs by the mean blanked control (CTRL) ODs for each sample, then multiplying by 100. The resulting % toxicity is provided as the "Data In" to the 4-Parameter (4P) curve fit, with the requested interpolation set to 50. The concentrations of the samples are calculated from the curve and the interpolated 50% value is provided.

Protocol name: IC50.prt

The protocol calls for an endpoint absorbance measurement at **620 nm**. Background absorbance of the microplate is removed by subtraction of blank wells. Two dose-response curves are plotted based on 6 sample-dilutions, which along with two controls are pipetted to the plate in triplicate.

Experiment File Name: IC50 (Toxicity) with data.xpt

The experiment file contains the absorbance measurements used to determine theoretical toxicity and to plot the dose-response curves.

Plate Configuration:

Two samples in dilutions of 10-60, each with a specific control and blank in triplicate.

Report:

The report contains a matrix with:
1. Well ID
2. Dilution value
3. Raw absorbance value
4. Calculated theoretical "Toxicity %."

The report also contains the two curves and their interpolations tables.

Kinetic Analysis β-Galactosidase with ONPG Substrate

Basis for the Assay:

The enzymatic product of the *LacZ* gene, β-galactosidase, catalyses the hydrolysis of β-D-galactosides, such as lactose, into their component sugars by hydrolysis of the terminal non-reducing β-D-galactose residues. Fortunately the substrate specificity of the enzyme is such that a variety of different substrates, each with a β-D-galactopyranoside moiety, can be acted upon. Investigators have taken advantage of this by synthesizing compounds which when hydrolyzed by β-galactosidase result in a colored product. One such compound is *o*-nitrophenol-β-D-galactoside (ONPG), which when hydrolyzed forms galactose and *o*-nitrophenol (ONP). The hydrolyzed product ONP absorbs light with a wavelength of 420 nm, while the substrate ONPG does not.

Protocol name: Kinetic β-Galactosidase Assay.prt

The protocol procedure includes a kinetic block with absorbance measurements taken at **420 nm** every 30 seconds for 30 minutes. The data reduction step is a kinetic well analysis to calculate the mean velocity (Mean V) from the data and plot the average for each substrate concentration. A 4-parameter logistic curve is used to describe the data.

Experiment File Name: Kinetic β-Galactosidase with data.xpt

The experiment file contains the absorbance measurements of the 61 kinetic reads. Well analysis data reduction generates the Mean V data set, which is used to plot the standard curve.

Plate Configuration:

The plate layout includes standards in duplicate with ONPG substrate concentrations ranging from 0 to 10 mM. Unknown samples in duplicate fill the rest of the plate.

Report:

A matrix report is defined to provide the kinetic curves and the mean V for each well. The standard curve is reported along with equation parameters and correlation coefficients.

Kinetic Endotoxin Chromogenic

Basis for the Assay:

Endotoxins are a class of compounds found in the outer layer of some gram-negative bacteria and are prevalent in the environment. Chromogenic endotoxin assays typically use a modified Limulus Amoebocyte Lysate (LAL) and a colored substrate to detect endotoxins. There are endpoint and kinetic methods for the assay. This sample describes a typical quantitative kinetic protocol wherein the intensity of color produced during the reaction is proportional to the amount of endotoxin in the wells. This protocol is set up to: a) find the concentration of endotoxin in samples by comparing the sample's Onset Time to the Onset Time of the standards and b) to calculate the amount of endotoxin recovered from sample controls (also known as "spikes" or "positive product controls") which have had a known concentration of endotoxin added to them initially.

Protocol name: Kinetic Endotoxin_Chromo.prt

The assay is run at 37^0 C, with a shake prior to the kinetic measurement. Kinetic measurements are taken in intervals of about 19 seconds for just over one hour at **405 nm**. Data Reduction steps include a Well Analysis to find the Onset OD (and Onset Time), Curve Analysis of (Onset Time v Concentration) and a Transformation formula to find the percent recovery of endotoxin in sample wells.

Experiment File Name: Kinetic Endotoxin Chromogenic.xpt

The experiment file contains the absorbance measurements used in the data reduction results to determine the Onset OD, the standard curve, and percent recovery of endotoxin.

Plate Configuration:

This sample layout includes 5 standards (50 to 0.005 eu/ml), each in triplicate. Samples 1 through 7 are in duplicate, as are the sample control wells. Samples 1-4 and sample controls 1-4 are diluted x 40.

Report:

This report includes a matrix containing the Well IDs, Onset Time and Percent Recovery. A second matrix includes the kinetic curves of al wells. The standard curve is presented as well as statistics tables for the standards, samples and sample control concentration results.

Lactate – NADH Assay

Basis for the Assay:

Lactic acid is a by-product of carbohydrate metabolism. Lactate is converted to pyruvate by the enzyme lactate dehydrogenase along with the deduction of NAD to NADH. NADH absorbs light at 340 nm, while NAD does not. Therefore this reaction can be followed by the increase in absorbance at 340 nm. Under conditions where NAD and Lactate dehydrogenase concentrations are in excess of lactate, the formation of NADH, along with 340 nm absorbance, is proportional to lactate levels. Thus 340 nm absorbance is directly proportional to lactic acid concentration.

Protocol name: Lactate-NADH.prt

The protocol procedure includes a single read step at **340 nm**. A linear regression curve fit is used to determine unknown concentrations.

Experiment File Name: Lactate – NADH with data.xpt

The experiment file contains the absorbance determination, which is used to plot the standard curve.

Plate Configuration:

The plate layout includes 12 standards in duplicate, in well positions A1 through B12. The standard concentrations range from 0 to 40 µg/ml. 36 unknown samples are defined in on the plate.

Report:

The standard curve is reported along with equation parameters and correlation coefficients. A matrix report is configured to provide the (1) 340 nm absorbance and (2) calculated lactate concentrations. A column type report containing the statistics for standards and samples is also provided.

Lowry Protein Assay

Basis for the Assay:

Quantification of total protein content is a measurement common to many applications in basic science and clinical research. The basis for this assay is the reduction of copper in alkali by protein (biuret reaction) in the presence of the chromogenic agent Folic phenol. This reaction produces a blue color with a very broad absorbance peak (600-850 nm).

Protocol name: Lowry Protein Assay.prt

The procedure includes a single read step at 660 nm. Data reduction includes a single curve fit step; Linear regression curve fit is used to determine unknown concentrations.

Experiment File Name: Lowry Protein Assay with data.xpt

The experiment file contains the absorbance determination, which is used to plot the standard curve.

Plate Configuration:

The plate layout includes standards in duplicate with concentrations from 0 to 200 µg/ml in wells A1 through B6. Samples in duplicate fill the rest of the plate.

Report:

The standard curve is reported along with equation parameters and correlation coefficients. A matrix report is configured to provide the (1) 660 nm absorbance and (2) calculated protein concentrations. Column type report containing the statistics for standards and samples is also provided.

Particulate Test A320

Basis for the Assay:

Purification procedures can often lead to the formation of insoluble particulates that remain after centrifugation. Absorbance values greater than 0.000 OD (after blanking) indicate the presence of particulates in nucleic acid samples.

Protocol name: Particulate Test A320.prt

The protocol calls for an absorbance measurement at 320 nm. Background absorbance of the microplate is removed by subtraction of a blank well. *Wells with a value of 0.010 or greater are considered to be positive for the presence of particulates.* This is expressed in a Cutoff data set.

Experiment File Name: Particulate Test A320.xpt

The experiment file does not contain any data.

Plate Configuration:

The plate layout places one blank in position A1. The rest of the wells contain 95 individual samples

Report:

The Report is configured to provide the (1) blanked 320 nm absorbance; (2) A Plate report using a cutoff of 0.010 indicating the possible presence (POS) or absence (NEG) of particulate is also reported.

Phenol Test

Basis for the Assay:

The presence of trace amounts of phenol can denature proteins and inhibit enzymatic reactions. The A_{260}/A_{270} ratio for nucleic acid sample should be greater than 1.0. Ratios less than 1.0 suggest the presence of trace amounts of phenol.

Protocol name: Phenol Test.prt

The procedure steps include a reading at 260 nm and 270 nm. Background absorbance of the microplate is removed by subtraction of a blank well. Using a Transformation the A_{260}/A_{270} ratio is calculated. *Wells with a ratio value of less than 1.0 are considered to be positive for the presence of phenol.* This is expressed in a Cutoff data set.

Experiment File Name: Phenol Test.xpt

The experiment data file does not contain any data

Plate Configuration:

The plate layout places one blank in position A1. The rest of the wells contain 95 individual samples.

Report:

Plate Report is configured to provide the (1) blanked 260 nm absorbance; (2) blanked 270 nm absorbance and (3) Calculated 260/270 ratio. A Plate report using a cutoff of 1.0 on the 260/270 ratio indicating the possible presence (POS) or absence (NEG) of EDTA is also reported.

Fluorescence

This section provides descriptions of the fluorescence protocol and experiment files shipped with Gen5, including those specific to Synergy 2.

AlamarBlue™ (Fluorescent)

Basis for the Assay:

The internal environment of proliferating cells is more reduced than that of non-proliferating cells. Compounds such as tetrazolium salts and AlamarBlue™, which can be reduced by cellular metabolic intermediates, can be used to monitor cellular proliferation. The reduction of AlamarBlue results in a measurable color shift, as well as the formation of a fluorescent molecule.

Protocol name: Alamar Blue-F.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **540/35** excitation filter and a **590/20** emission filter.

Experiment file name: Alamar Blue-F.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration provides for samples to be run in duplicate. In addition, two blank wells are defined on the plate. In regards to data reduction, only blank well subtraction has been programmed.

Report:

The report is configured to provide the (1) raw fluorescence, and (2) blanked fluorescence. The monitoring of AlamarBlue reduction by fluorescence is usually expressed as relative fluorescence emission units (RFU) as a function of the time of incubation.

Alexa Fluor® 488

Basis for the Assay:

Alexa Fluor® 488 is a member of the Alexa Fluor series of fluorescent dyes manufactured and sold by Molecular Probes (Invitrogen). These dyes are reported to be brighter, have better photostability, have a greater pH range and water solubility, as well as have improved labeling chemistry than many conventional fluorescent compounds. Alexa Fluor 488 would be a replacement for fluorescein in regards to excitation and emission wavelengths.

Protocol name: AlexaFluor 488.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : AlexaFluor 488.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration does not provide any sample or standard information. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, controls).

Report:

The report is configured to provide the raw fluorescence.

AttoPhos®

Basis for the Assay:

Enzyme linked immunosorbent assays (ELISA) have been used to quantitate a wide range of compounds and pathogens for almost 40 years. One of the most commonly used enzyme conjugates is Alkaline Phosphatase (AP). (2'-[2-benzothiazoyl]-6'-hydroxybenzothiazole phosphate (BBTP) serves as a substrate for AP. Unreacted BBTP is weakly fluorescent, but in the presence of alkaline phosphatase it is converted to a highly fluorescent product, BBT, which has excitation and emission maxima of 435 nm and 575 nm respectively. ELISA assays that employ an alkaline phosphatase conjugate can be used with AttoPhos® substrate system.

Protocol name: Attophos.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **440/30** excitation filter and a **560/40** emission filter.

Experiment File Name : Attophos with Data.xpt

The experiment data file contains data from several unknown alkaline phosphatase samples using AttoPhos as the substrate.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. The plate includes seven AP standards ranging from 0 to 0.05 DEA Units/well, and two blank wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) blanked fluorescence, (3) calibration curve, (4) curve fitting results, and (5) unknown sample concentrations.

Calcium Green

Basis for the Assay:

Detection of Calcium (Ca^{+2}) ions is most often accomplished by using sensing probes or dyes. These dyes typically consist of a fluorophore and a region that confers analyte specificity, such a BAPTA group for Ca^{+2}. The multiple anionic carboxyl groups bind the divalent calcium ion in a similar fashion as the calcium specific chelator, ethylene glycol bis(β-aminoethylether ether)-*N,N,N',N'*-tetraacetic acid (EGTA). The indicator dye Calcium Green increases its fluorescence in the presence of calcium. The increase in fluorescence is proportional to the calcium ion concentration.

Protocol name: Calcium Green.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter

Experiment File Name : Calcium Green with Data.xpt

The experiment data file contains all of the fluorescence determinations for a calcium determination using calcium green dye.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of calcium concentrations (0 to 39 μM) available from Molecular Probes' (Invitrogen) calcium calibrator kit. There are no blanks or controls. Thus 37 samples can be run on a microplate in duplicate in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated calcium concentrations of unknown samples.

Caspase-3 AMC Substrate

Basis for the Assay:

Caspase proteins are proteolytic enzymes involved in programmed cell death or Apoptosis. This proteolytic processing occurs at critical aspartic acid residues that conform to the caspase recognition sequence. Different caspase enzymes recognize related but different protein sequences. Caspase-3 can be monitored using a synthetic peptide, which releases a fluorometric moiety when cleaved by the enzyme. Prior to cleavage the substrate is non-fluorescent. The increase in fluorescence is proportional to the amount of caspase-3 activity present in the sample.

Protocol name: Caspase-3 AMC Substrate.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **360/40** excitation filter and a **460/40** emission filter.

Experiment File Name : Caspase-3 with Data.xpt

The experiment data file contains all of the fluorescence determinations for a Caspase-3 enzyme activity determination using a peptide substrate with an AMC fluorescent moiety.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of Caspase-3 enzyme activities (0 to 1.6 ng/well). The plate includes 8 standard concentrations and two blank wells, but no controls. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) blanked fluorescence, (3) standard curve, and (4) calculated caspase-3 concentrations of unknown samples.

Caspase-3 (Assay Design)

Basis for the Assay:

Caspase proteins are proteolytic enzymes involved in programmed cell death or Apoptosis. This proteolytic processing occurs at critical aspartic acid residues that conform to the caspase recognition sequence. Different caspase enzymes recognize related but different protein sequences. Caspase-3 can be monitored using a synthetic peptide, which releases a fluorometric moiety when cleaved by the enzyme. Prior to cleavage the substrate is non-fluorescent. The increase in fluorescence is proportional to the amount of caspase-3 activity present in the sample. In this assay kit, the control wells, with a precisely determined amount of activity, are used to generate a conversion factor that allows conversion of the nominal activity of the standards to an actual measured activity.

Protocol name: Caspase-3 (Assay Design).prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **360/40** excitation filter and a **460/40** emission filter

Plate file name: Caspase-3.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of Caspase-6 enzyme activities (6.25 to 100 U/well). The plate includes 5 standard concentrations, two blank wells, and two control wells. Thus 41 samples can be run on a microplate in duplicate in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) blanked fluorescence, (3) standard curve, (4) results of the conversion factor, and (5) calculated caspase-3 concentrations of unknown samples.

Caspase-6 (Assay Design)

Basis for the Assay:

Caspase proteins are proteolytic enzymes involved in programmed cell death or Apoptosis. This proteolytic processing occurs at critical aspartic acid residues that conform to the caspase recognition sequence. Different caspase enzymes recognize related but different protein sequences. Caspase-6 can be monitored using a synthetic peptide, which releases a fluorometric moiety when cleaved by the enzyme. Prior to cleavage the substrate is non-fluorescent. The increase in fluorescence is proportional to the amount of caspase-6 activity present in the sample. In this assay kit, the control wells, with a precisely determined amount of activity, are used to generate a conversion factor that allows the conversion the nominal activity of the standards to an actual measured activity.

Protocol name: Caspase-6 (Assay Design).prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **360/40** excitation filter and a **460/40** emission filter

Experiment File Name : Caspase-6.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of Caspase-6 enzyme activities (3.13 to 50 U/well). The plate includes 5 standard concentrations, two blank wells, and two control wells. Thus, 41 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) blanked fluorescence, (3) standard curve, (4) results of the conversion factor, and (5) calculated caspase-6 concentrations of unknown samples.

Catalase (Amplex Red)

Basis for the Assay:

Catalase enzyme catalyzes the conversion of hydrogen peroxide to water. Amplex® Red, when present of with horseradish peroxidase enzyme, reacts with H_2O_2 in a 1:1 stiochiometry to produce resorufin, a red fluorescent compound, which has an absorption and fluorescence emission maxima of 563 nm and 587 nm respectively. Therefore the presence of catalase would be expected to reduce the amount of fluorescence produced from a constant amount of HRP and H_2O_2 present in a well. The amount of catalase present is proportional to the change (reduction) in fluorescence generated.

Protocol name: Catalase.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **540/35** excitation filter and a **590/20** emission filter. The data reduction uses a transformation to subtract the value of each well from the mean of the 0 mU/ml catalase standard (STD1). This difference in fluorescence is then plotted against known catalase concentrations in order to provide a calibration curve.

Experiment File Name : Catalase (Amplex Red).xpt

The experiment data file contains all of the fluorescence determinations for a catalase enzyme activity determination using Amplex Red as the substrate.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of catalase enzyme activity (0 to 1000 U/ml) as defined by Invitrogen's Catalase detection kit. The plate includes 8 standard concentrations, but no blank or controls wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, (3) calculated catalase concentrations and statistics of unknown samples.

CBQCA

Basis for the Assay:

Quantitation of total protein content is a measurement common to many applications in basic science and clinical research. The compound, 3-(4-carboxybenzoyl) quinoline -2-carboxaldehyde (CBQCA) reacts with primary amines of proteins to form fluorescent moieties. This compound has the added advantage of functioning well in the presence of lipids, which normally interfere with protein determinations. The fluorescent intensity is directly proportional to the total protein concentration present in the sample.

Protocol name: CBQCA.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **460/40** excitation filter and a **560/40** emission filter.

Experiment File Name : CBQCA with Data.xpt

The experiment data file contains all of the fluorescence determinations for a CBQCA total protein determination using BSA as the protein.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of protein (0 to 1000 ng/well) as defined by Invitrogen's CBQCA detection kit. The plate includes 7 standard concentrations, but no blank or controls wells. Thus, 41 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated catalase concentrations and statistics of unknown samples.

Cholesterol (Amplex Red)

Basis for the Assay:

Cholesterol esterase is used to hydrolyze any cholesterol ester present to free cholesterol. Free cholesterol is converted to cholest-4-ene-3-one ketone product by the action of cholesterol oxidase and in doing so generates hydrogen peroxide (H_2O_2). Amplex red reagent and H_2O_2 are converted to resorufin and water in a one-to-one stiochiometry by horseradish peroxidase. Resorufin is a highly fluorescent compound with absorption maxima of 563 nm and a peak emission wavelength of 587 nm. The amount of fluorescence produced is directly proportional to the amount of total cholesterol (free and esterified) present in the sample.

Protocol name: Cholesterol (Amplex Red).prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **540/35** excitation filter and a **590/20** emission filter.

Experiment File Name : Cholesterol with Data.xpt

The experiment data file contains all of the fluorescence determinations for a Cholesterol determination using Invitrogen's Cholesterol determination kit.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of Cholesterol concentration (0 to 10 µM) as defined by Invitrogen's Cholesterol determination kit. The plate includes 8 standard concentrations, but no blank or control wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, (3) calculated cholesterol concentrations and statistics of unknown samples.

CyQuant® Cell Proliferation Assay

Basis for the Assay:

CyQuant Cell Proliferation Assay is a means to directly quantitate the density of cells in culture. CyQuant Cell Proliferation assay uses a proprietary dye, CyQuant GR, which becomes fluorescent when bound to cellular nucleic acids. Cells are lysed by the addition of lysis buffer containing the dye and the samples are quantitated directly. A reference standard curve using specific numbers of cells can be created that allows converting fluorescence into cell number. Alternatively cellular DNA can be directly quantitated using a DNA only standard curve.

Protocol name: CyQuant Cell Proliferation Assay.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Plate file name: CyQuant Cell Proliferation Assay.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that lysed and RNase treated samples will be run in duplicate. The plate includes nine different DNA concentrations of standards ranging from 0 to 1000 ng/mL. Thus, 39 samples can be run in duplicate.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) DNA concentrations of unknown samples.

DAPI

Basis for the Assay:

DAPI (4',6'-diamidino-2-phenylindole) is a DNA binding fluorescent dye the exhibits blue fluorescence. DAPI stain associates in the minor groove of dsDNA, preferentially binding to AT-base clusters. Binding to DNA results is an approximate 20-fold enhancement in fluoresescence.

Protocol name: DAPI.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **360/40** excitation filter and a **460/40** emission filter.

Experiment File Name : DAPI.xpt

The plate data file does not contain any data.

Plate Configuration:

Plate configuration includes two blank wells and 94 unknown samples. The only data reduction performed is blank subtraction.

Report:

The report is configured to provide the (1) raw fluorescence and (2) blanked fluorescence.

EGFP

Basis for the Assay:

Fluorescent proteins are used as traceable genetic elements for a number of different cellular experiments. Fluorescent proteins are peptides that are inherently fluorescent and do not require any cofactors or modifications. EGFP is the brightest and most commonly used of the fluorescent proteins. EGFP is a variant that has had its genetic sequence modified to have excitation and emission wavelengths closer to that of fluorescein, as well as reflect human cells' codon usage bias. EGFP, when translated, has an excitation and emission maxima of 488 nm and 509 nm respectively. Gene expression, in particular, is most easily performed in the microplate format.

Protocol name: EGFP.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter. Because EGFP experiments usually employ cells, fluorescent measurements are made from the bottom.

Experiment File Name : EGFP.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. The plate includes two blank wells, but no standards or controls wells. Thus, 47 samples can be run on a microplate in duplicate. The only data reduction performed is blank subtraction.

Report:

The report is configured to provide (1) blanked fluorescence.

Enzchek® Amylase

Basis for the Assay:

α-Amylase is the catalytic enzyme responsible for the hydrolysis of starch into smaller components such as maltose and dextrins. Quantitation of α-Amylase is important in the diagnosis of pancreatitis and diabetes, as well as, a number of different industrial applications. The test is based on the digestion of a starch substrate that has been labeled with BODIPT FL dye to such an extent that the fluorescence is quenched. When digestion of the substrate by the enzyme occurs the quenching is relieved, resulting in the release of fluorescent product.

Protocol name: Enzchek Amylase Assay.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Plate file name: Enzchek Amylase Assay.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. The plate includes six different concentrations of standards ranging from 0 to 200 mU/ml. Thus, 42 samples can be run.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) Amylase concentrations of unknown samples.

Ethidium Bromide

Basis for the Assay:

Ethidium bromide is a DNA binding fluorescent dye the exhibits red fluorescence. Ethidium bromide stain intercalates into the minor groove of dsDNA, showing little or no sequence preferences. Binding to DNA results is an approximate 10-fold enhancement in fluorescence.

Protocol name: Ethidium Bromide.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **540/35** excitation filter and a **620/40** emission filter.

Experiment File Name : Ethidium Bromide.xpt

The plate data file does not contain any data.

Plate Configuration:

Plate configuration includes two blank wells and 94 unknown samples. The only data reduction performed is blank subtraction.

Report:

The report is configured to provide (1) blanked fluorescence.

FAM

Basis for the Assay:

FAM (fluorescein) is a fluorescent compound widely used for oligonucleotide labeling. Labeled oligonucleotides have been used as detection probes for real time PCR (RTPCR), as well as primers for DNA PCR reactions and DNA sequencing.

Protocol name: FAM.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : FAM.xpt

The plate data file does not contain any data.

Plate Configuration:

Plate configuration provides for samples to be run in duplicate. In addition, two blank wells are defined on the plate. In regards to data reduction, only blank well subtraction has been programmed.

Report:

The report is configured to provide the (1) raw fluorescence and (2) blanked fluorescence.

Fluorescamine Protein Quantitation

Basis for the Assay:

Quantitation of total protein content is a measurement common to many applications in basic science and clinical research. Fluorescamine reacts with primary amines of proteins to form a fluorescent moiety. This compound reacts very rapidly, but is poorly soluble in water, requiring it to be dissolved in an organic solvent prior to reacting with protein. The fluorescence intensity is directly proportional to the total protein concentration present in the sample.

Protocol name: Fluorescamine.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **400/30** excitation filter and a **460/40** emission filter.

Experiment File Name : Fluorescamine with Data.xpt

The experiment data file contains all of the fluorescence determinations for a fluorescamine total protein determination using BSA as the protein.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of protein (0 to 600 µg/ml). The plate includes 7 standard concentrations, two blank wells, but no controls wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) blanked fluorescence data, (3) standard curve, and (4) calculated protein concentrations and statistics of unknown samples.

Glucose (Amplex Red)

Basis for the Assay:

Cholesterol oxidase reacts with D-glucose to form D-gluconolactone and hydrogen peroxide (H_2O_2). Amplex red reagent and H_2O_2 are converted to resorufin and water in a one-to-one stiochiometry by horseradish peroxidase. Resorufin is a highly fluorescent compound with absorption maxima of 571 nm and a peak emission wavelength of 585 nm. The amount of fluorescence produced is directly proportional to the amount of D-glucose present in the sample.

Protocol name: Glucose (Amplex Red).prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **540/35** excitation filter and a **590/20** emission filter.

Experiment File Name : Glucose (Amplex Red).xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of D-glucose concentration (0 to 125 µM) as defined by Invitrogen's Glucose/glucose oxidase Assay kit. The plate includes 8 standard concentrations, but no blank or controls wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, (3) calculated cholesterol concentrations and statistics of unknown samples.

Hoechst 33258 High Concentrations

Basis for the Assay:

Bisbenzimide stain, also known as Hoechst 33258, binds to dsDNA molecules and markedly increases in fluorescence when it does so. The dye, weakly fluorescent itself in solution, binds specifically to the A-T base pairs in dsDNA resulting in an increase in fluorescence and a shift in the emission maximum from 500 to 460 nm. In the presence of high salt (2M NaCl) the stain is specific for dsDNA, with little to no interference from ssDNA or RNA. The fluorescence intensity is directly proportional to the total nucleic acid concentration present in the sample.

Protocol name: Hoechst 33258 High Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **360/40** excitation filter and a **460/40** emission filter.

Experiment File Name : Hoechst 33258 (High Concentrations) with Data.xpt

The experiment data file contains all of the fluorescence determinations for a Hoechst 33258 stain dsDNA determination.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a dsDNA standard curve (0 to 20 μg/ml). The plate includes 7 standard concentrations, two blank wells, but no controls wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) blanked fluorescence, (3) standard curve, and (4) calculated dsDNA concentrations and statistics of unknown samples.

Hoechst 33258 Low Concentrations

Basis for the Assay:

Bisbenzidine stain, also known as Hoechst 33258, binds to dsDNA molecules and markedly increases in fluorescence when it does so. In the presence of high salt (2M NaCl) the stain is specific for dsDNA, with little to no interference from ssDNA or RNA. The fluorescence intensity is directly proportional to the total nucleic acid concentration present in the sample.

Protocol name: Hoechst 33258 Low Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **360/40** excitation filter and a **460/40** emission filter.

Experiment File Name : Hoechst 33258 (Low Concentrations) with Data.xpt

The experiment data file contains all of the fluorescence determinations for a Hoechst 33258 stain dsDNA determination.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a dsDNA standard curve (0 to 400 ng/ml). The plate includes 8 standard concentrations, but no blank or control wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated dsDNA concentrations and statistics of unknown samples.

Hydrogen Peroxide (Amplex Red)

Basis for the Assay:

Several enzymatic reactions produce hydrogen peroxide (H_2O_2) as a by-product, while other reactions consume the molecule. Regardless of the reaction, Amplex® Red reagent can be utilized to measure H_2O_2 levels. Amplex® Red, in the presence of peroxidase enzyme, reacts with H_2O_2 in a 1:1 stiochiometry to produce resorufin, a red fluorescent compound. Resorufin has an absorption and fluorescence emission maxima of 563 nm and 587 nm respectively. The amount of fluorescence produced is directly proportional to the amount of hydrogen peroxide originally present in the sample.

Protocol name: Hydrogen peroxide (Amplex Red).prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **540/35** excitation filter and a **590/20** emission filter.

Experiment File Name : Hydrogen peroxide with Data.xpt

The experiment data file contains all of the fluorescence determinations for a hydrogen peroxide determination using Invitrogen's Hydrogen Peroxide Assay kit.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of Cholesterol concentration (0 to 1.25 µM) as defined by Invitrogen's Hydrogen peroxide assay kit. The plate includes 8 standard concentrations, but no blank or controls wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, (3) calculated cholesterol concentrations and statistics of unknown samples.

NanoOrange®

Basis for the Assay:

Quantitation of total protein content is a measurement common to many applications in basic science and clinical research. Proteins are heat denatured prior to reacting with NanoOrange compound diluted in an SDS containing buffer. The binding of the NanoOrange compound with protein results in the formation of a fluorescent moiety. Because the SDS maintains the proteins in a denatured state, plates can be read up to six hours after reaction. The fluorescence intensity is directly proportional to the total protein concentration present in the sample.

Protocol name: NanoOrange.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **595/35** emission filter.

Experiment File Name : NanoOrange with Data.xpt

The experiment data file data contains all of the fluorescence determinations for a NanoOrange total protein determination using BSA as the protein.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of protein (0 to 10 µg/ml). The plate includes 11 standard concentrations, but has no blanks or controls wells. Thus, 37 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated protein concentrations and statistics of unknown samples.

OliGreen® High Concentrations

Basis for the Assay:

OliGreen stain binds to ssDNA and oligonucleotide molecules and markedly increases in fluorescence when it does so. The stain is specific for ssDNA, but will show some increase in fluorescence in the presence of RNA. The fluorescence intensity is directly proportional to the total nucleic acid concentration present in the sample.

Protocol name: OliGreen High Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : OliGreen (High Concentrations) with Data.xpt

The experiment data file contains all of the fluorescence determinations for an OliGreen oligonucleotide determination.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a dsDNA standard curve (0 to 1,000 ng/ml). The plate includes 5 standard concentrations, but has no blanks or controls wells. Thus, 43 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated protein concentrations and statistics of unknown samples.

OliGreen® Low Concentrations

Basis for the Assay:

OliGreen stain binds to ssDNA and oligonucleotide molecules and markedly increases in fluorescence when it does so. The stain is specific for ssDNA, but will show some increase in fluorescence in the presence of RNA. The fluorescence intensity is directly proportional to the total nucleic acid concentration present in the sample.

Protocol name: OliGreen Low Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : OliGreen (Low Concentrations).xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a dsDNA standard curve (0 to 50,000 pg/ml). The plate includes 5 standard concentrations, but has no blanks or controls wells. Thus, 43 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated dsDNA concentrations and statistics of unknown samples.

OPA Protein Quantitation

Basis for the Assay:

Quantitation of total protein content is a measurement common to many applications in basic science and clinical research. The compound, σ-phthaldialdehyde (OPA) in conjunction with reduced sulfhydryl groups reacts with primary amines to form fluorescent moieties. This compound reacts very rapidly, but is much more water-soluble than fluorescamine. The fluorescence intensity is directly proportional to the total protein concentration present in the sample.

Protocol name: OPA.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **360/40** excitation filter and a **460/40** emission filter.

Experiment File Name : OPA with Data.xpt

The experiment data file contains all of the fluorescence determinations for an OPA total protein determination using BSA as the protein.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a standard curve of protein (0 to 1000 µg/ml). The plate includes 8 standard concentrations, but has no blanks or controls wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated protein concentrations and statistics of unknown samples.

ORAC

Basis for the Assay:

The oxygen radical absorbance capacity (ORAC) assay is a means to quantify the antioxidant potential of foods. The ORAC assay depends on the free radical damage to a fluorescent probe, such as fluorescein, to result in a downward change of fluorescent intensity. The presence of antioxidants results in an inhibition in the free radical damage to the fluorescent compound. This inhibition is observed as a preservation of the fluorescent signal. Reactions containing antioxidants and blanks are run in parallel using equivalent amounts of a ROS generator and fluorescent probe. One can quantitate the protection by calculating the area under the curve (AUC) from the experimental sample. After subtracting the AUC for the blank, the resultant difference would be the protection conferred by the antioxidant compound. Comparison to a set of known standards allows one to calculate equivalents and compare results from different samples and experiments.

Protocol name: ORAC no injection.prt (FL Reader with temperature control)

The protocol calls for a 30-minute incubation at 37 °C to allow components to equilibrate to the correct temperature. The reaction is then initiated by injecting 25 µl of AAPH solution manually (plate is ejected) and a kinetic fluorescence measurement is performed at 37°C using a **485/20** excitation filter and a **528/20** emission filter pair. The entire plate is read every minute for 60 minutes. The raw data for each kinetic read is normalized to the initial measurement using a kinetic formula, with the resultant referred to as "formula result." The net AUC is calculated by subtracting the blank wells from the standards and samples. The net AUC for the standards is then plotted and the curve fit used to calculate the ORAC of unknown samples.

Experiment File Name : ORAC no injection.xpt

The experiment file does not contain data.

Plate Configuration:

Plate configuration assumes that samples will be run in triplicate and the outer wells on all sides are not being used for measurement. Included is a Trolox standard curve (0 to 100 µM). The plate includes 6 standard concentrations, six blank wells and one control sample run in triplicate. Thus, 11 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the Net AUC in matrix format, the standard curve, and the calculated ORAC values and statistics of unknown samples.

ORAC with Injection

Basis for the Assay:

The oxygen radical absorbance capacity (ORAC) assay is a means to quantify the antioxidant potential of foods. The ORAC assay depends on the free radical damage to a fluorescent probe, such as fluorescein, resulting in a downward change of fluorescent intensity over time. The presence of antioxidants results in an inhibition in the free radical damage to the fluorescent compound. This inhibition is observed as a preservation of the fluorescent signal. Reactions containing antioxidants and blanks are run in parallel using equivalent amounts of a ROS generator and fluorescent probe. One can quantitate the protection by calculating the area under the curve (AUC) from the experimental sample. After subtracting the AUC for the blank, the resultant difference would be the protection conferred by the antioxidant compound. Comparison to a set of known standards allows one to calculate equivalents and compare results from different samples and experiments. The assay requires the temperature equilibration of the fluorescent probe and antioxidant compounds prior to the initiation of the assay. The reaction is initiated by the addition of an oxidizing compound such as 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH). The use of the injector allows for the equilibration, as well as the precise timing of the fluorescent measurements for all the wells after the initiation of the assay.

Protocol name: ORAC with Injection.prt (Fluorescence Reader with injectors and temperature control)

The protocol calls for a 30-minute incubation at 37 °C to allow components to equilibrate to the correct temperature. The reaction is then initiated by injecting 25 µl of AAPH solution followed by kinetic fluorescence measurements at 37°C using a **485/20** excitation filter and a **528/20** emission filter pair. The entire plate is read every minute for 60 minutes. The raw data for each kinetic read is normalized to the initial measurement using a kinetic formula, with the resultant referred to as "formula result". The net AUC is calculated by subtracting the blank wells from the standards and samples. The net AUC for the standards is then plotted and the curve fit used to calculate the ORAC of unknown samples.

Experiment File Name : ORAC with injection with data.xpt

The experiment data file contains all of the fluorescence determinations for an ORAC determination.

Plate Configuration:

Plate configuration assumes that samples will be run in triplicate and the outer wells on all sides are not being used for measurement. Included is a Trolox standard curve (0 to 100 µM). The plate includes 6 standard concentrations, six blank wells and one control sample run in triplicate. Thus, 11 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the Net AUC in matrix format, the standard curve, and the calculated ORAC values and statistics of unknown samples.

PicoGreen® High Concentrations

Basis for the Assay:

PicoGreen stain binds to dsDNA molecules and markedly increases in fluorescence when it does so. The stain is specific for dsDNA, showing limited increase in fluorescence in the presence of ssDNA or RNA. The fluorescence intensity is directly proportional to the dsDNA present in the sample.

Protocol name: PicoGreen High Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : PicoGreen (High Concentrations) with Data.xpt

The experiment data file contains all of the fluorescence determinations for a PicoGreen dsDNA determination.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a dsDNA standard curve (0 to 1,000 ng/ml). The plate includes 5 standard concentrations, but has no blanks or controls wells. Thus, 43 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated protein concentrations and statistics of unknown samples.

PicoGreen® Low Concentrations

Basis for the Assay:

PicoGreen stain binds to dsDNA molecules and markedly increases in fluorescence when it does so. The stain is specific for dsDNA, showing limited increase in fluorescence in the presence of ssDNA or RNA. The fluorescence intensity is directly proportional to the dsDNA concentration present in the sample.

Protocol name: PicoGreen Low Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : PicoGreen (Low Concentrations) with Data.xpt

The experiment data file contains all of the fluorescence determinations for a PicoGreen dsDNA determination.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a dsDNA standard curve (0 to 25,000 pg/ml). The plate includes 5 standard concentrations, but has no blanks or controls wells. Thus, 43 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated dsDNA concentrations and statistics of unknown samples.

Propidium Iodide

Basis for the Assay:

Propidium iodide (PI) binds to DNA by intercalating between bases with little or no sequence specificity. Propidium Iodide stain is weakly fluorescent, but when bound to DNA molecules it increases in fluorescent intensity approximately 20-30 fold. The fluorescence intensity is directly proportional to the total nucleic acid concentration present in the sample.

Protocol name: Propidium Iodide.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **645/40** emission filter.

Experiment File Name : Propidium Iodide with Data.xpt

The experiment data file contains all of the fluorescence determinations for a Propidium Iodide, dsDNA determination.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a dsDNA standard curve (0 to 2.5 µg/ml). The plate includes 7 standard concentrations, two blank wells, but no controls wells. Thus, 40 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) blanked fluorescence, (3) standard curve, and (4) calculated dsDNA concentrations and statistics of unknown samples.

Quanta Blu™

Basis for the Assay:

Enzyme linked immunosorbent assays (ELISA) have been used to quantitate a wide range of compounds and pathogens for almost 40 years. One of the most commonly used enzyme conjugates is horseradish peroxidase (HRP). QuantaBlu™ serves as a substrate for HRP. Unreacted Quanta Blu is nonfluorescent, but in the presence of hydrogen peroxide (H_2O_2) and HRP it is converted to a fluorescent product, which has excitation and emission maxima of 325 nm and 420 nm respectively. ELISA assays that employ an HRP conjugate can be used with Quanta Blu substrate.

Protocol name: Quanta Blu.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a (320/20) excitation filter and a (420/50) emission filter.

Experiment file name: Quanta.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. The plate includes two blank wells, and no controls wells. A standard curve based on the analyte in question is required.

Report:

The report is configured to provide the (1) Blanked fluorescence.

Resorufin

Basis for the Assay:

Resorufin is the product of a number of commercially available enzyme substrates (e.g. Amplex Red). It has an absorption and fluorescence emission maxima of 563 nm and 587 nm respectively. The amount of resorufin present is proportional to the amount of fluorescence generated.

Protocol name: Resorufin.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **540/35** excitation filter and a **590/20** emission filter. The protocol is configured such that the correct filters are selected, but the user is required to provide the remainder of the plate layout, data reduction are any subsequent reports.

Experiment File Name : Resorufin.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate and includes two blank wells. Thus, 47 samples can be run on a microplate in duplicate.

Report:

The report is configured to provide the (1) raw fluorescence and (2) blanked fluorescence.

RiboGreen® High Concentrations

Basis for the Assay:

RiboGreen stain binds to RNA. With binding the stain's fluorescence increases markedly. The stain is not necessarily specific for RNA, but will also bind ssDNA and dsDNA molecules. The fluorescence intensity is directly proportional to the nucleic concentration present in the sample.

Protocol name: RiboGreen High Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : RiboGreen (High Concentrations) with Data.xpt

The experiment data file contains all of the fluorescence determinations for a RiboGreen RNA determination.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a RNA standard curve (0 to 1,000 ng/ml). The plate includes 5 standard concentrations, but has no blanks or controls wells. Thus, 43 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated protein concentrations and statistics of unknown samples.

RiboGreen® Low Concentrations

Basis for the Assay:

RiboGreen stain binds to RNA. With binding the stain's fluorescence increases markedly. The stain is not necessarily specific for RNA, but will also bind ssDNA and dsDNA molecules. The fluorescence intensity is directly proportional to the nucleic concentration present in the sample.

Protocol name: RiboGreen Low Concentrations.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20** excitation filter and a **528/20** emission filter.

Experiment File Name : RiboGreen (Low Concentrations).xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration assumes that samples will be run in duplicate. Included is a RNA standard curve (0 to 50 ng/ml). The plate includes 5 standard concentrations, but has no blank or control wells. Thus, 43 samples can be run on a microplate in duplicate, in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated RNA concentrations and statistics of unknown samples.

ROX

Basis for the Assay:

ROX (carboxy-X-rhodamine) is a fluorescent compound widely used for oligonucleotide labeling. Labeled oligonucleotides have been used as detection probes for real time PCR (RTPCR), as well as primers for DNA PCR reactions and DNA sequencing.

Protocol name: ROX.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **575/15** excitation filter and a **635/32** emission filter.

Experiment File Name : ROX.xpt

The plate data file does not contain any data.

Plate Configuration:

Plate configuration provides for samples to be run in duplicate. In addition, two blank wells are defined on the plate. In regards to data reduction, only blank-well subtraction has been programmed.

Report:

The report is configured to provide the (1) raw fluorescence and (2) blanked fluorescence

TAM

Basis for the Assay:

TAM or TAMRA (tetramethylrhodamine) is a fluorescent compound widely used for oligonucleotide labeling. Labeled oligonucleotides have been used as detection probes for real time PCR (RTPCR), as well as primers for DNA PCR reactions and DNA sequencing.

Protocol name: TAM.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **560/15** excitation filter and a **590/20** emission filter.

Experiment File Name : TAM.xpt

The plate data file does not contain any data.

Plate Configuration:

Plate configuration provides for samples to be run in duplicate. In addition, two blank wells are defined on the plate. In regards to data reduction, only blank well subtraction has been programmed.

Report:

The report is configured to provide the (1) raw fluorescence and (2) blanked fluorescence.

Tryptophan Quantitation

Basis for the Assay:

Aromatic amino acids, such as Tryptophan can be measured using fluorescence. Ultra-violet light from the Xenon flash lamp can be used to excite tryptophan molecules in solution or in peptides and proteins. The monochromator is used to select excitation wavelength, while a standard long bandpass filter is used for emission. The increase in fluorescence is proportional to the amount of tryptophan present in the sample.

Protocol name: Tryptophan Quantitation.prt (TRF capable Fluorescence Reader)

The protocol calls for a fluorescence measurement using the flash lamp (TRF mode) at **280/10** excitation wavelength with a **340/30** emission filter. The reading parameter options include a 0 µsec delay before data collection and a 100 µsec collection time.

Experiment File Name : Tryptophan Quantitation with Data.xpt

The experiment data file contains all of the fluorescence determinations for a Tryptophan amino acid determination.

Plate Configuration:

Plate configuration provides for samples to be run in duplicate. Included is a standard curve of Tryptophan concentrations (0 to 1000 ng/ml). The plate includes 8 standard concentrations, but no blanks or controls. Thus 40 samples can be run on a microplate in duplicate in addition to the standard curve.

Report:

The report is configured to provide the (1) raw fluorescence, (2) standard curve, and (3) calculated Tryptophan concentrations of unknown samples.

Synergy 2: G Factor Determination with Fluorescein (Fluorescence Polarization)

Basis for the Assay:

Gen5, by default, uses a G Factor in the Fluorescence Polarization (FP) calculation to normalize the polarization value obtained on fluorescein to 20 mP (known reference value of unbound fluorescein). BioTek tested numerous readers in the factory to select a default G Factor value of .87 and this is used in the system-generated FP calculation. This sample protocol is provided to assist you in determining the specific G factor for your reader.

The G factor corrects for the variations resulting from differences between instruments with different optical designs. Using a G factor is not required when measuring fluorescence polarization, but it will correct some hardware bias that varies from one instrument design to another. This experiment allows you to calculate the value of the G factor when using fluorescein as the fluorescent label.

Protocol name: G Factor Determination with Fluorescein.prt (Fluorescence Reader)

The protocol calls for a fluorescence measurement using a **485/20 nm** excitation filter and a **528/20 nm** emission filter.

Experiment file name: G Factor Determination with Fluorescein.xpt

The experiment file contains an example of data and G factor calculation.

Plate Configuration:

The plate layout only uses 2 columns of a 96 well plate. Column 3 is filled with buffer and is defined as 8 blank wells, column 5 is filled with 200 µl of a 1 nM solution of sodium fluorescein (8 replicates).

Report:

This experiment files does not include a pre-set report. The calculated value of the G factor can be displayed in the matrix view of the plate: select the "**G factor calculation**" data set in the Plate Views Data field drop-down list.

Synergy 2: TRF Cyclic AMP Delfia (Time Resolved Fluorescence)

Basis for the Assay:

cAMP quantification is commonly used in primary or secondary high-throughput screening for G-protein coupled receptors. Several different assay technologies can be used to quantify cAMP, including fluorescence polarization, time-resolved fluorescence (TRF) and time-resolved fluorescence resonance energy transfer (TR-FRET). This sample file provides an example of TRF determination of cAMP using a Delfia® kit from PerkinElmer® on the Synergy 2 multi-detection microplate reader.

Protocol name: TRF Synergy 2 Cyclic AMP Delfia.prt

The protocol calls for a time resolved fluorescence measurement using a **340/30 nm** excitation filter and a **620/40 nm** emission filter, or **360/40 nm** excitation filter and a **620/40 nm** emission filter. The two different excitation wavelengths are provided as an example of different setups that would work. Only one excitation filter is required to run this assay.

Experiment file name: TRF Synergy 2 Cyclic AMP Delfia with data.xpt

The experiment file contains an example of data coming from a series of standards, a control and blank wells.

Plate Configuration:

10 standards are dispensed across the plate, in replicates of 8. Column 11 contains a control in 8 replicates, and column 12 contains 8 blanks.

Report:

The report includes the standard curve and curve fitting results table, statistics on the standard replicates and the plate layout, raw data measurements and calculated concentrations in the matrix format.

Synergy 2: LANCE TR-FRET Cyclic AMP

Basis for the Assay:

cAMP quantification is commonly used in primary or secondary high-throughput screening for G-protein coupled receptors. Several different assay technologies can be used to quantify cAMP, including fluorescence polarization, time-resolved fluorescence (TRF), and time-resolved fluorescence resonance energy transfer (TR-FRET). This sample file provides an example of TR-FRET determination of cAMP using a LANCE™ kit from PerkinElmer® on the Synergy 2 multi-detection microplate reader.

Protocol name: LANCE TR-FRET cAMP.prt

The protocol calls for a time-resolved fluorescence measurement using a **330/80 nm** excitation filter and a **665/10 nm** emission filter. The time-resolved parameters are 50 μs delay and 200 μs integration time.

Experiment file name: LANCE TR-FRET cAMP with data.xpt

The experiment file contains an example of data coming from a series of standards.

Plate Configuration:

11 standards are dispensed across the plate, in replicates of 16, in a 384-well plate.

Report:

The report includes a standard curve and statistics on the standard replicates.

Synergy 2: Polarization with Fluorescein Label (Fluorescence Polarization)

Basis for the Assay:

Fluorescence Polarization is used extensively in research and screening laboratories. It can provide information on changes in molecular mobility found, for example, in receptor–ligand interactions, protein–DNA interactions, or membrane fluidity assays. It consists in two successive fluorescence measurements through a parallel and a perpendicular polarizer. The two results are automatically compared and polarization is reported. Any small fluorescent molecule can be used to design a polarization assay, and fluorescein is one of the most commonly used labels.

Protocol name: Polarization with Fluorescein Label.prt (Fluorescence Reader)

The protocol calls for a measurement using a 485/20 nm excitation filter and a 528/20 nm emission filter. Switching from the parallel to the perpendicular polarizer is automatic. The result of this Read step is two sets of raw data and calculation of polarization values based on the raw data.

Experiment file name: Polarization with Fluorescein Label with Data.xpt

The experiment file contains an example of simulated data.

Plate Configuration:

The plate map contains 8 standards in duplicate, two blanks, and samples in duplicate.

Report:

The report includes a standard curve and statistics on the standard and sample replicates.

Synergy 2: AlphaScreen cAMP

Basis for the Assay:

cAMP quantification is commonly used in primary or secondary high-throughput screening for G-protein coupled receptors. Several different assay technologies can be used to quantify cAMP, including fluorescence polarization, time-resolved fluorescence resonance energy transfer (TR-FRET) and AlphaScreen. This sample file provides an example of determination of cAMP using an AlphaScreen® kit from PerkinElmer® on the Synergy 2 multi-detection microplate reader.

Protocol name: AlphaScreen.prt

The AlphaScreen assay measurement requires a specific sequence of events: excite the sample, turn the excitation light off, then measure the delayed emission. On the Synergy 2, this is achieved as follows: a first filter set is used for the excitation phase (680/30 nm - Plug). This filter set does not generate useful data, and is only used to excite the sample. A second filter set (Plug – 570/100 nm) allows to block the excitation light (role of the plug) and measure the delayed emission coming from the sample.

Experiment file name: AlphaScreen cAMP with data.xpt

The experiment file contains an example of data coming from a series of standards. CAMP concentration is determined from the standard curve.

Plate Configuration:

The plate map contains 15 standards in replicates of 8, in a 384-well plate.

Report:

The report includes the raw data of delayed emission (Plug/570/100 nm) in matrix format, the standard curve and statistics on the standard and sample replicates and summary listing of the Procedure steps.

Luminescence

This section provides descriptions of the luminescence protocol and experiment files shipped with Gen5.

Dual-Luciferase® Reporter (DLR) Gene Assay

Basis for the Assay:

Genetic reporting assays are used to study gene expression and cellular responses to external stimuli. Dual-reporter assays use two independent reporter systems simultaneously to improve experimental accuracy. One reporter is usually tied to measuring the response resulting from the experimental conditions and is often referred to as the "experimental" reporter. The other reporter is designed such that it does not respond to the experimental conditions, acting as an internal control from which data generated by the experimental reporter can be normalized to. Normalization of the data serves to compensate for variability caused by differences in transfection efficiency, cell viability, cell lysis, and pipetting. This assay requires automated injection and luminescence detection. "Flash" assays are typically more sensitive than "Glow" assays (see Dual-Glo™ Luciferase Reporter Gene Assay), but provide a lower throughput.

Protocol name: DLR Assay.prt (Luminescence Reader with injectors)

The protocol executes the following sequence of steps:

1. Inject firefly luciferase substrate (100 µl)
2. Wait two seconds
3. Read sample for 10 seconds (Read 1)
4. Inject Renilla luciferase substrate (100 µl)
5. Wait two seconds
6. Read sample for 10 seconds (Read 2)

A ratio (Read 1 / Read 2) is calculated for each well. Results available at the end of the process are: raw data and DLR ratio.

Experiment file name: DLR Assay.prt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains samples only, in duplicate. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, blanks, controls).

Report:

The report provides:

1. A matrix containing the raw data and DLR ratio
2. Statistics on sample replicates

Dual-Glo™ Luciferase Reporter Gene Assay

Basis for the Assay:

Genetic reporting assays are used to study gene expression and cellular responses to external stimuli. Dual-reporter assays use two independent reporter systems simultaneously to improve experimental accuracy. One reporter is usually tied to measuring the response resulting from the experimental conditions and is often referred to as the "experimental" reporter. The other reporter is designed such that it does not respond to the experimental conditions, acting as an internal control from which data generated by the experimental reporter can be normalized to. Normalization of the data serves to compensate for variability caused by differences in transfection efficiency, cell viability, cell lysis, and pipetting. This assay requires luminescence detection. "Glow" assays are typically less sensitive than "Flash" assays (see **Dual-Luciferase® Reporter (DLR) Gene Assay**), but don't require automated injection and have a higher throughput.

Protocol name: Dual Luciferase Glow (Dual Glo).prt (Luminescence Reader)

The protocol executes the following sequence of steps:
1. Read plate, 1 second/well (Read 1)
2. Eject plate carrier and display message (add Renilla substrate)
3. Read plate, 1 second/well (Read 2)

A ratio (Read 1 / Read 2) is calculated for each well. Results available at the end of the process are: raw data and Firefly/Renilla ratio (read 1/read 2 ratio).

Experiment file name: Dual Luciferase Glow (Dual Glo).xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains samples only, in duplicate. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, blanks, controls).

Report:

The report provides:
1. A matrix containing the raw data and Firefly/Renilla ratio
2. Statistics on sample replicates

Flash Luciferase Assay

Basis for the Assay:

Reporter genes, such as luciferase, are important tools for studying gene expression. The use of reporter genes allows the *in vitro* and *in vivo* measurement of gene expression from virtually any endogenous genetic control element. Luciferase enzyme and its subsequent luminescent reaction is often the gene reporter of choice for many experimental conditions. This assay requires automated injection and luminescence detection. "Flash" assays are typically more sensitive than "Glow" assays (see Glow Luciferase Assay), but provide a lower throughput.

Protocol name: Luciferase Flash.prt (Luminescence Reader with injectors)

This protocol executes the following process:
1. Injection of the trigger reagent (100 µl)
2. Wait two seconds
3. Read sample for 5 seconds

Experiment file name: Luciferase Flash.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains samples only. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, blanks, controls).

Report:

The report provides:
1. A summary of the procedure
2. The plate layout
3. Statistics on sample replicates

Glow Luciferase Assay

Basis for the Assay:

Reporter genes, such as luciferase, are important tools for studying gene expression. The use of reporter genes allows the *in vitro* and *in vivo* measurement of gene expression from virtually any endogenous genetic control element. Luciferase enzyme and its subsequent luminescent reaction is often the gene reporter of choice for many experimental conditions. "Glow" assays are typically less sensitive than "Flash" assays (see Flash Luciferase Assay), but don't require automated injection and have a higher throughput.

Protocol name: Luciferase Glow.prt (Luminescence Reader)

This protocol is an end-point luminescence measurement integrating each well for 1 second.

Experiment file name: Luciferase Glow.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains samples in duplicate and a Blank well in duplicate. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, controls).

Report:

The report provides:
1. The plate layout and raw data in a matrix format
2. Statistics on replicates

Flash ATP Assay

Basis for the Assay:

All living things utilize ATP as a means for storing metabolic energy. Because of this, the detection and quantitation of ATP can be used as a means to detect and/or quantitate microorganisms such as bacteria and somatic cells. A very common assay relies on the ATP-dependence of the firefly luciferase reaction to detect live organisms. This assay requires automated injection and luminescence detection. "Flash" assays are typically more sensitive than "Glow" assays (see **Glow ATP Assay**), but provide a lower throughput.

Protocol name: ATP Flash.prt (Luminescence Reader with injectors)

This protocol automates the following process:
1. Injection of the trigger reagent (100 µl)
2. Wait two seconds
3. Read sample for 5 seconds

Experiment file name: ATP Flash.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains samples only, in duplicate. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, blanks, controls).

Report:

The report provides:
1. A summary of the procedure
2. The plate layout
3. Statistics on sample replicates

Glow ATP Assay

Basis for the Assay:

All living things utilize ATP as a means for storing metabolic energy. Because of this, the detection and quantitation of ATP can be used as a means to detect and/or quantitate microorganisms such as bacteria and somatic cells. A very common assay relies on the ATP-dependence of the firefly luciferase reaction to detect live organisms. "Glow" assays are typically less sensitive than "Flash" assays (see **Flash ATP Assay**), but don't require automated injection and have a higher throughput.

Protocol name: ATP Glow.prt (Luminescence Reader)

This protocol is an end-point luminescence measurement integrating each well for 1 second.

Experiment file name: ATP Glow.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains samples in duplicate and a Blank well in duplicate. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, controls).

Report:

The report provides:
1. The plate layout, Well IDs, and blanked results
2. Statistics on sample replicates

DsDNA Assay

Basis for the Assay:

Accurate determination of DNA concentration in a sample is important for many different procedures in molecular biology. Luminescent-based detection of dsDNA is a sensitive and specific way to detect linear dsDNA including PCR fragments. Plasmid and chromosomal DNA can be quantitated following linearization. The measurement is based upon a series of coupled enzymatic reactions that produce a light signal proportional to the amount of linear DNA in a sample. This assay requires automated injection and luminescence detection.

Protocol name: dsDNA Quantitation.prt (Luminescence Reader with injectors)

This protocol executes the following process:
1. Injection of the trigger reagent (100 μl)
2. Wait three seconds
3. Read sample for 1 second

Results available at the end of the process are: raw data, blanked data, and the calculated DNA concentration of samples based on the standard curve.

Experiment file name: dsDNA quantitation.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration provides for samples to be run in duplicate. Included are five standards, a blank, and 42 samples, in duplicate.

Report:

The report provides:
1. The plate layout, Well IDs, and blanked results
2. Standard curve with curve fit information
3. Statistics on blanks, standards and samples

Fast Kinetic Luminescence

Basis for the Assay:

Some luminescent assays have extremely fast kinetic signals, and it is sometimes useful to measure the kinetic of the signal after injection rather than just integrate it for a few seconds. The obtained kinetic curve can provide more detailed information than an end-point integration. This assay requires automated injection and luminescence detection.

Protocol name: Fast Kinetic Luminescence.prt (Luminescence Reader with injectors)

This protocol executes the following process:
1. Injection of the trigger reagent (100 µl)
2. Measure luminescence kinetically for 10 seconds with a 20 ms interval (50 data points/second)

Results available at the end of the process are: raw data, kinetic curves, kinetic analysis (Min/Max analysis).

Experiment file name: Fast Kinetic Luminescence.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains only samples, in duplicate. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, controls).

Report:

The report provides:
1. Matrix of kinetic curves
2. Well zoom examples
3. Statistics on samples

Long Kinetic Luminescence

Basis for the Assay:

Cell-based assays often require following the change in signal over some period of time. This assay performs a plate-mode kinetic measurement with temperature control.

Protocol name: Long Kinetic Luminescence.prt (Luminescence Reader with temperature control)

This protocol executes the following process:

1. Bring reading chamber to 37ºC before starting the read process
2. Measure luminescence kinetically for 2 hours with a 5-minute interval, each measurement is a 1 second integration

Results available at the end of the process are: raw data, kinetic curves, kinetic analysis: Maximum slope (Max V).

Experiment file name: Long Kinetic Luminescence.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration contains samples only, in duplicate. It may have to be updated with more specific information (e.g., standards to generate a calibration curve, controls).

Report:

The report provides:
1. Matrix of kinetic curves
2. Well zoom examples
3. Statistics on samples

Qualitative ELISA Glow Luminescence

Basis for the Assay:

ELISA assays are an extremely common way to detect biomolecules in complex samples. Most ELISA assays use absorbance as a detection method. In some cases, when the concentrations to be measured are extremely low, luminescence provides more sensitivity and is used instead of absorbance. The assay principle remains exactly the same, the only difference being the use of a luminescent substrate instead of a colorimetric one at the end of the process.

Protocol name: Qualitative ELISA Glow Luminescence.prt (Luminescence Reader)

This protocol executes a 1-second read per well in luminescence mode.

Results available at the end of the process are: raw data, blanked data, and qualitative interpretation based on a cut-off calculation.

Experiment file name: Qualitative ELISA Glow Luminescence.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration includes a blank well, positive control, negative control and 45 samples, all in duplicate.

Report:

The report provides:
1. Plate layout and qualitative interpretation (Positive/Negative) in a matrix format
2. Statistics on sample duplicates.

Quantitative ELISA Glow Luminescence

Basis for the Assay:

ELISA assays are an extremely common way to detect biomolecules in complex samples. Most ELISA assays use absorbance as a detection method. In some cases, when the concentrations to be measured are extremely low, luminescence provides more sensitivity and is used instead of absorbance. The assay principle remains exactly the same, the only difference being the use of a luminescent substrate instead of a colorimetric one at the end of the process.

Protocol name: Quantitative ELISA Glow Luminescence.prt (Luminescence Reader)

This protocol executes a 1-second read per well in luminescence mode. Results available at the end of the process are: raw data, blanked data, standard curve, calculated sample concentration.

Experiment file name: Quantitative ELISA Glow Luminescence.xpt

The experiment data file does not contain any data.

Plate Configuration:

Plate configuration includes a blank well, a standard curve (0.5 to 5000 pg/ml) based on 6 standards, and 41 samples, all in duplicate.

Report:

The report provides:
1. Plate layout and calculated concentrations in a matrix format
2. Standard curve
3. Statistics on duplicates

Synergy 2: MycoAlert Assay

Basis for the Assay:

Mycoplasma contamination is a common problem with continuously cultured cell lines. The MycoAlert® assay from Cambrex is a biochemical test that screens for the presence of mycoplasma-specific enzymes that interact with reagents catalyzing the conversion of ADP to ATP. By measuring the ATP produced before and after the addition of a substrate, a ratio is obtained which indicates the presence or absence of mycoplasma.

Protocol name: MycoAlert.prt

This procedure includes a 5-minute incubation in the reader, a first 1-second per well integration, then a "plate eject" command. This command allows the manual addition of the MycoAlert substrate. The plate is then incubated for ten minutes in the reader before a second read is performed. The protocol calculates the MycoAlert ratios, interprets the results (positive, negative, re-test) and plots a standard curve.

Experiment file name: MycoAlert with data.xpt

The file incorporates data from a serial dilution of a positive control.

Plate Configuration:

Plate configuration includes a serial dilution of a positive control. 9 dilutions of the control are available, each dilution in replicates of 8.

Report:

The report lists the Procedure and Data Reduction steps, and includes:
1. Standard curve and curve fitting results table
2. Plate layout and interpreted results (Cutoffs) in a matrix format
3. Plate layout and calculated concentrations in a matrix format