Gen5™ & Gen5 Secure User's Guide

Microplate Data Collection & Analysis Software

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Chapter 3

Essential Concepts

This section reveals the basic concepts upon which Gen5 was built. Learning them will enhance your experience using Gen5.

Essential Concepts

Understanding the basic concepts behind Gen5's structure and behavior will help you make the most of it. A few topics are covered here, on the next few pages, more information is provided in Gen5's Help.

- Experiment vs. Protocol on page 45
- **File Formats:**

  Gen5's files are identified by their filename extension:

  - .prt = Protocol file
  - .xpt = Experiment file (contains the protocol and any data acquired or generated within the experiment)
- File Storage on page 47
- Best Practices on page 48

In Gen5, select Help>Help Topics to find:

- **Multiple-Plate Experiments**
- **Security and FDA Electronic Records Compliance**

❖ Only the **Gen5 Secure** level of software offers all the capability required to meet the FDA's electronic records requirements: §21 CFR Part 11.

Experiment vs. Protocol

Gen5™ uses two common terms to define distinct elements of its toolkit. The distinction is subtle and understanding it will improve your Gen5 experience.

Protocol (*.prt)	Experiment (*.xpt)
A protocol is a "recipe" or set of instructions designed to capture, transform and report and/or export data	An experiment has a copy of the protocol and at least one plate. It executes the instructions provided by the protocol to produce results
Protocols are created and saved as standalone files. They function as a template; an unlimited number of experiments can be based on one	While an experiment is created using an existing protocol, the experiment's copy of the protocol can be modified within the experiment
A protocol consists of reading requirements, like detection method and wavelength, and reading-related actions, like shaking and incubation (Procedure), plate layout, data reduction, and data viewing, reporting and exporting definitions	Running an experiment is the only way to process a protocol. Gen5's Quick Read function may at first appear to skip the protocol development stage, but it uses the default protocol, and generally requires reading parameters to be defined
A protocol can be used repeatedly (as-is or modified) within experiments. By itself, a protocol does not produce results. Protocols do not have plates associated with them	Multiple plates can be processed in an experiment; each one considered a unique assay with independently reported or exported results. The exception is multi-plate protocols, described later
.prt is the protocol's filename extension	.xpt is the experiment's filename extension
A copy of the protocol is saved within an experiment, or as a standalone .prt file. Since protocols do not have plates, they cannot generate data outside of an experiment	An experiment is saved as the full collection of procedures, formulas, reporting definitions, and other details, i.e., the protocol, and the plate data from readings and calculation results
The Gen5™ Secure level of software maintains an audit trail of all activity and changes related to a protocol. All other Gen5™ software levels do not support this feature	Data acquired and transformed in an experiment is protected by an audit trail in both Gen5 Secure and other Gen5 software editions. The Reader Control edition does not support this feature

Protocol (*.prt)	Experiment (*.xpt)
Changes made to a standalone protocol are not reflected in any previously created experiments based on that protocol. A new experiment must be created to apply the revised protocol	Within an experiment, you can select **Save Protocol As** to capture the current details of the protocol and save them as either a new protocol or as an overwrite of the original protocol

- All newly created protocols and (unless another protocol is selected) experiments are based upon the **Default Protocol**

- Gen5™ also supports more complex multi-plate protocols that are not covered in this introductory material. Check out: Designing a Multi-Plate Protocol in a subsequent chapter.

About File Storage

File Types

Gen5™ creates two file types: Protocol = .prt and Experiment = .xpt

- The Gen5 executable file (.exe) and numerous other types of supporting files, like an Excel® template, are also installed on the computer.
- In addition, **Clarity™ Microplate Luminometer** users will work with Clarity protocol files, which use a .BPF extension. Gen5 references the Clarity files as they contain the reading parameters required to control the luminometer.

Databases

Gen5 installs two databases on your system called LocalDB and SharedDB. While the databases are always used for critical, internally-used files, Gen5 offers you the choice of using the Windows® File System or the Gen5 (SharedDB) database for storing Gen5's Protocol (.prt) and Experiment (.xpt) files. This option, combined with the ability to create multiple databases, allows you to structure file storage according to your organization's requirements.

- Files may be stored on the computer's hard drive, on a network, or on a CD or other portable medium. Windows Explorer or a similar application can be used to view the file names and locations, and to move, copy, rename, and delete files.
- Alternatively, protocol and experiment files may be stored in a secure, shared-access database. This database, initially named SharedDB.mdb, can be stored on a user's computer or on a shared-access network/computer (LAN). Gen5 provides a special file maintenance utility for viewing the file names and their locations, and for moving, copying, renaming, deleting, importing, and exporting files.
- Select the preferred method of storing protocol and experiment files at **System>Preferences>File Storage**

File Location

During a conventional installation:

- the program files are stored in this default location: C:\Program Files\BioTek\Gen5 *(software edition)*
- the databases are stored in these default locations:
  Windows XP and 2000 systems: C:\Documents and Settings\All Users\Application Data\BioTek Instruments\Gen5 *(software edition)**(version #)*\SharedDB or LocalDB
  Windows Vista: Windows XP and 2000 operating systems: C:\Program Data\BioTek \Gen5 *(software edition)**(version #)*\SharedDB or LocalDB
- Gen5 installs Protocol and Experiment folders in the respective File Storage locations, e.g. C:\Program Files\BioTek\Gen5 *(software edition)*\Protocol

Best Practices

Like most software tools, Gen5™ is flexible, it offers several ways to accomplish a task. Here are some recommendations for saving time and using it most efficiently:

Efficiencies

- For an assay or experiment that you will run numerous times, develop a **Protocol** to define the Procedure, Data Reduction, Data Views and Reports required. Then you can run an Experiment (File>New Experiment) based on the Protocol whenever necessary. You can fine-tune the protocol within an experiment, but remember to select File>Save Protocol As to update the original protocol with your improvements.
- Just like word processing documents, when you run similar types of experiments, you can use File>Save As to give you a head start creating a new protocol based an existing protocol that contains the same plate layout, reading parameters, or other elements that will be repeated in your new protocol.
- Define and customize Data Views before selecting what to include in reports or export files. All the on-screen data (i.e. data views) can be reported or exported. If you use on-screen views and paper reports equally, it is most efficient to first fine-tune the Data Views, and then include them in reports/exports.
- Always assign **Blanks** to the plate. Blanks can be deionized (DI) water, buffer, reagent without analyte, substrate and so on. When running fluorescence cellular assays, a DI-water blank illustrates the background contributed by the instrument and labware as separate from the cells and media. Identify the location of the Blanks in the Plate Layout and Gen5 will automatically create the blank-subtraction data reductions.
- Backup your database regularly, BioTek recommends once per week for most organizations. If you're using Gen5's Database for protocol and experiment file storage, use the built-in Periodic Optimization feature.
- Take action if you get a warning message about the remaining size of your databases, see Maintaining Files for instructions on reducing the database size.
- Consider using Gen5's automatic Save feature to create a new, date-stamped folder for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 will keep all that data organized by date. Define this kind of file management setting in the Default Protocol so it will apply to all newly created protocols.
- Turn off the Multi-Read Calculation option to improve Gen5's performance. Calculation results will be the same, but your PC's resources will not be diverted for performing interim calculations.

Time Savers

- **Partial Plate**: for assays using strips or partially-filled plates, especially if the read steps are long or complicated, you can save time by telling the reader exactly which wells or portion of the plate to read
- **Default Protocol**: all newly created protocols and Quick Reads are based on the Default Protocol. If some protocol elements, like plate layout, runtime prompts, report headers and footers, etc., are largely the same for most of your projects, you'll save significant time by defining these elements before creating the next protocol/experiment
- **Print Preview**: save time and paper by viewing reports on-screen before sending them to the printer

Chapter 4

Assay Examples

This section contains step-by-step instructions for programming commonly known assays using Gen5. Gen5 also ships with Sample Protocols for most of these assays. Both the Sample Protocols and the assay descriptions are learning tools. It is your responsibility to customize and validate them to meet your needs.

Sample Protocols and Experiments

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, we recommend selecting **File>Save As** after opening them and assigning 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 available: Absorbance, Fluorescence, Luminescence and for Synergy 2 users, there is a Synergy 2 folder within each detection method folder:

- 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
- Gen5's **Welcome** screen also offers the option to open a **Sample File**

> ❖ **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 the Samples Protocol Listing to see if your reader is compatible with the defined steps.

Sample Protocols and Experiments Guide

You can review a complete description of samples in the Sample Protocols and Experiments Guide.PDF shipped with Gen5: click the Windows® **Start**, select **All Programs**>**Gen5**>**Sample Protocols and Experiments Guide**

> ❖ Gen5 installs a copy of the Sample Protocols and Experiments Guide in the Samples folder of the main Gen5 directory. (By default this is C:\Program Files\BioTek\Gen5\Samples)

You can also find a summary listing and brief description of the sample protocols in Gen5's Help. Review the description of the sample protocol to make sure it is compatible with your reader.

How do I set up my assay?

Here are step-by-step instructions for creating Gen5 Protocols to run common assays. (More Assay Examples can be found in Gen5's Help.) We hope that by following the instructions, making some changes to names and other details, you can adapt them for use in your lab. Also see the **Kinetic Analysis** chapter.

Absorbance

Fluorescence

Luminescence

Dispensing Reagent

Quantitative ELISA Example

To help you set up your own assay here is an example of the steps required to run a quantitative ELISA assay. In this example we set up an endpoint Absorbance read, subtract Blank wells from all others, plot a standard curve, and define a Control to express the samples as a percentage of the control.

It may be easier to follow these instructions if you have already watched the Gen5 Basic series of online tutorials: select Help>Tutorials or if you've completed the learning exercises described in the Getting Started Guide.

To set up the protocol, we'll define the:

1. Reading Procedure
2. Plate Layout
3. Data Reductions

❖ Reporting Results is the same process for all types of experiments

1. Defining the reading Procedure

This assay example has the simplest read Procedure: a single-wavelength Absorbance endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and select the wavelength. Use the drop-down list or type the wavelength in the text field (overwrite the current value).
4. Click **OK** twice to save the Procedure

2. Defining the Plate Layout

This step is critical for the data reduction steps to be defined later. Here's the plate layout we need:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	BLK	CTL1	CTL1	CTL1	SPL8	SPL8	SPL8	SPL16	SPL16	SPL16
B	STD1 0	STD1 0	STD1 0	SPL1	SPL1	SPL1	SPL9	SPL9	SPL9	SPL17	SPL17	SPL17
C	STD2 10	STD2 10	STD2 10	SPL2	SPL2	SPL2	SPL10	SPL10	SPL10	SPL18	SPL18	SPL18
D	STD3 20	STD3 20	STD3 20	SPL3	SPL3	SPL3	SPL11	SPL11	SPL11	SPL19	SPL19	SPL19
E	STD4 30	STD4 30	STD4 30	SPL4	SPL4	SPL4	SPL12	SPL12	SPL12	SPL20	SPL20	SPL20
F	STD5 40	STD5 40	STD5 40	SPL5	SPL5	SPL5	SPL13	SPL13	SPL13	SPL21	SPL21	SPL21
G	STD6 50	STD6 50	STD6 50	SPL6	SPL6	SPL6	SPL14	SPL14	SPL14	SPL22	SPL22	SPL22
H	STD7 60	STD7 60	STD7 60	SPL7	SPL7	SPL7	SPL15	SPL15	SPL15	SPL23	SPL23	SPL23

The critical factor is using the Well IDs, not their location on the plate. We did not need to customize the Well IDs for this example. We simply selected the Type, defined the known concentration of the standards and assigned them to the plate:

Well ID	Type	Description
BLK	Blank	DI water only
STD	Standard	Known concentrations
CTL1	Assay Control	Known Control
SPL	Sample	Unknown samples

Find specific instructions in the Preparing Plates chapter.

3. Defining the Data Reduction Steps

Now that we've defined the reading parameters and plate layout, we can define the data reduction steps: blank-well subtraction, standard curve, and expressing samples as a percentage of the control. Gen5 creates the blank-subtraction step for you automatically.

1. Select **Protocol> Data Reduction**
   Notice that one Transformation, named "Blank nnn" where nnn is the wavelength, has already been created. We'll use the results of this calculation to plot the standard curve.
2. Click **Curve Analysis**

3. Notice on the **Data In** tab, the **Well ID** is set to STD and X Axis Data to <Plate Layout Settings>. The known concentrations entered for Standards are plotted on the X Axis. Use the drop-down list for the **Y Axis Data** to select Blank *nnn (wavelength)*
4. Click the **Curve Fit** tab: depending on your assay, you may want to change the curve fit method to 4 Parameters or another option, or use Log values on the X or Y axis. For now, retain the defaults and click the **Data Out** tab. Take note that the **Data Set Name** produced from the standard curve is called Conc (by default. You can change it.). Click OK to save and close the curve.
5. Click **Transformation**
    1. For the **Data In** use the drop-down list to select Conc.
    2. Enter a **New Data Set Name** for the results of this calculation, e.g. %Control
    3. In the **Formula** field enter: (X / CTL1)*100
    Retain the default setting to Use single formula for all wells. X represents the value of the current well. CTL1 is the well ID for the control we assigned in Plate Layout.

 After the plate is read, you can return to the Data Reduction dialog to make any needed changes, like the Curve Fit Method. Do not change the Data Out or Data Set Names, this would invalidate the data reduction steps that use those data sets.

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6. Save the protocol.

Now you're ready to define your reporting requirements, and run the protocol in an experiment.

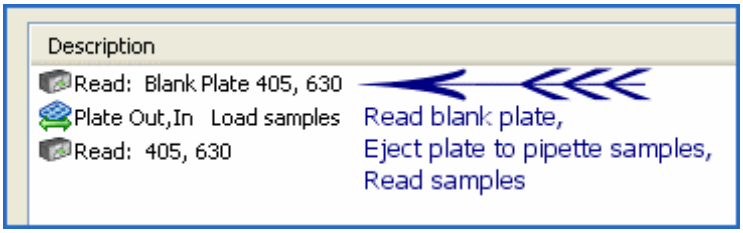
Subtracting Blank Plate Reads

To perform a blank-plate subtraction in your experiment, set up an additional **Read Step** for the blank plate, and then, create a **Data Reduction Transformation** to subtract the measurements of the blank plate from the samples plate.

You can insert a **Plate in/Out** step in the **Procedure** sequence to first read the blank plate, pause the experiment to pipette samples to the plate, and then, read the samples plate.

Step-by-step procedure:

1. Select **File>New Protocol**
2. Double click **Procedures** to set the reading parameters:
    1 First, create a **Read Step** for the blank plate: enter **Blank** for the Step Label to easily identify the raw data.



    2 Add a **Plate In/Out** step to eject the plate to pipette samples, standards, etc. Optionally, enter "Load Samples" in the comment field.

    3 Finally, create a **Read Step** for the samples plate.

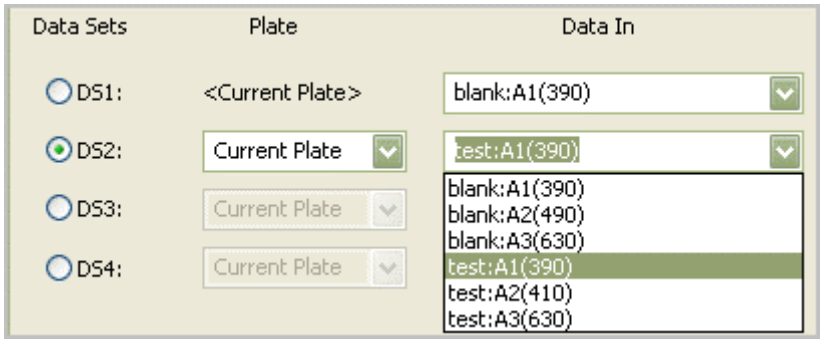
> ❖ Other steps can be included in the sequence, like Set Temperature and Shake, if required.

3. Set up the **Plate Layout** to match the distribution of samples and standards or controls.
4. Double click **Data Reduction** to define a transformation: Blank Plate Data Reduction
    1 The dialog will contain any automatically generated data reductions. Highlight the top-most one and click **Transformation**, to position the blank subtraction as the first calculation.

    2 Click 

    3 In the **Multiple Data Sets** screen, use the drop-down lists to select the **Blank** plate read data for **DS1** and the samples plate data for **DS2**.

> ❖ In multiple wavelength protocols there may be several data sets to choose from. If you've used the **Step Labels** for each Read Step, it's easy to match up the blank plate with the samples plate.



4 Enter a **New Data Set Name** for the resulting data set, e.g. Blanked 390

5 Enter **DS2-DS1** in the **Plate Formula** field and click OK.

6 Repeat steps 2-4 to create as many blank-plate subtraction Transformations as needed, e.g. one per wavelength.

7 Now you're ready to create other Data Reduction steps using the blanked data sets. For example, select Curve Analysis to generate a standard curve based on the blank-subtracted test plate.

5. Customize the Data Views and fine-tune the Report Builder as needed before saving and closing this protocol. **File>Save**

   Now you're ready to run the protocol in an experiment: **File> New Experiment**

Running the experiment:

After reading the blank plate, Gen5 ejects the carrier so you can load the samples

If you entered a comment, e.g. Load Samples in the Plate In / Out step, it is displayed on screen. Here's how to proceed:

- Click OK **after loading the samples**, when you're ready to continue reading the plate

Pathlength Correction Example

Here is an example of the steps required to perform pathlength correction in an ELISA assay. In this example we set up an endpoint Absorbance read, subtract Blank wells from all others, and transform the data to determine the concentrations of the unknown samples. This is the process used to create the Direct Oligo Quantification assay shipped as a sample protocol with Gen5.

It may be easier to follow these instructions if you have already watched the Gen5 Basic series of online tutorials: select Help>Tutorials or if you've completed the learning exercises described in the Getting Started Guide.

To set up this protocol, we'll define the:

1. Reading Procedure
2. Plate Layout
3. Data Reductions

❖ **Reporting Results** is the same process for all types of experiments

1. Defining the reading Procedure

This assay example has the simplest read Procedure: a single-wavelength Absorbance endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and keep the default settings for Detection Method, Read Type and Read Speed
4. Fill in the checkbox next to **Pathlength Correction**. [Optionally, click the 3-dot button to view (and modify if desired) the test and reference wavelengths used in the process.]
5. Set the **Wavelength**. Use the drop-down list or type the wavelength in the text field (overwrite the current value). For this example, enter 260.
6. Click **OK** twice to save the Read step and the Procedure

2. Defining the Plate Layout

For this assay example, the plate layout is very simple, comprising two blank wells and 94 unknown samples:

1. Select **Protocol> Plate Layout**

2. In the **Well Settings** box, select the **Type** of specimen, first Blanks, then Samples
3. Assign the blanks to cells A1 and B1
4. Change the **Type** to Sample, make sure **Next ID** is enabled, click and drag over the remaining wells to assign the unknown samples

3. Defining the Data Reduction Steps

After defining the reading parameters and plate layout, we can define the data reduction steps. Gen5 creates the blank subtraction and the pathlength correction for you automatically.

1. Select **Protocol> Data Reduction**
   Notice the two Transformations, "Blank 260" and "Corrected [Blank 260]", Gen5 first subtracts the blanks and then applies the Pathlength Correction Calculation.
2. Click **Transformation** to add anther Data Reduction step
3. For the **Data In** use the drop-down list to select **Corrected [Blank 260]** data set
4. Enter a **New Data Set Name** for the results of this calculation, e.g. Concentration
5. In the **Plate Formula** field enter: **X*32.5**
   Retain the default setting to Use single formula for all wells. X represents the value of the current well. 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.
6. Save the protocol.

Now you're ready to define your reporting requirements, and run the protocol in an experiment.

Dual Wavelength Absorbance Endpoint

Here are step-by-step instructions for setting up a dual-wavelength absorbance read with known concentrations of standards against which a linear regression curve is plotted.

Create the protocol:

1. Select **File > New Protocol**
2.  Double-click **Procedure** in the menu tree:
   - Click **Read** to set the reading parameters: Keep the default settings for Detection Method and Read Type: Absorbance Endpoint
   - For **Wavelengths**, click the button for **2** and use the drop-down list to select (or enter) the test and reference wavelengths: 410 and 630 for this example.
   - Click **OK** twice to close the Read Step, and then, the Procedure dialogs.
3.  Double-click **Plate Layout** to define the location of standards, samples, and blanks on the microplate. For this example, the standards are placed in the center of the plate, modify the instructions to match the distribution of samples and standards on your plate:
   - Set the Well Settings Type to **Standard** and click the 3-dot button next to the **Conc.** field to enter the expected concentrations. For this example, leave 0 in the STD1 cell at the top of the table. Select **Incr.** with a tick mark, and enter 10 in the field, then click in the **STD1** cell, then in the STD2 cell, and each subsequent cell in the table until STD8. Click **OK** to save and close the concentrations.
   - At the grid, set the Number of **Replicates** to 2, and select **Next Conc.** under **Auto Select**. Click and hold as you roll the mouse over the **5** and **6** columns, (the cursor changes to a black, down-facing arrow) to fill the entire columns.
   - Set the Well Settings Type to **Blank**, keep the Number of **Replicates** at 2, and click and drag over wells A1 and A2.
   - Set the Well Settings Type to **Sample**, keep the Number of **Replicates** at 2, and select **Next ID** under **Auto Select**. Click and drag the cursor over the remaining wells in columns 1 and 2, and then 3-4, and then 7-12, to assign samples to all the other wells of the plate.
4.  Double-click **Data Reduction**
   Gen5 automatically creates the Blank-Subtraction transformations.
   - Click **Transformation** to set up the calculation:
5. Click 

6. For **DS1** (selected by default) use the drop-down list to select **Blank 410**
7. Select **DS2** and use the drop-down list to select **Blank 630**
8. Click **OK** to close the **Multiple Data Set** dialog
9. For this example, we'll call the **New Data Set Name**: **Dual Wavelength**. Enter the name in the text box. Dual wavelength is also known as **Delta OD**, you may want to use this name instead .
10. In the **Plate Formula** field enter: **DS1-DS2** to subtract the reference wavelength (630) measurements from the test (410) measurements. Retain the default: Use single formula for entire plate.
11. Click **OK** to save and close the **Transformation**
    - Click **Curve Analysis**. In the Data In tab, use the drop-down list to select **Y Data: Dual Wavelength** and click **OK**.

❖ For this simple protocol, the remaining default settings are acceptable. More options are available, like customizing the names of data sets, plotting interpolations in the generated curve, and so on. See **Plotting a Curve** in the Data Reduction Options chapter.

    - Click **OK** to close the Data Reduction dialog.
12. Set the **Report** parameters and **Data Views** as desired. For instructions, see Viewing Results.
13. Save the protocol: select **File>Save** and name it **DualWave1** for this example.

Run the protocol:

Now, you're ready to run the DualWave1 protocol in an experiment.

1. Select **File>New Experiment**. By default, Gen5 highlights the DualWave1 protocol in the dialog, making selection quick and easy.
2. If the reader is all set up, you're ready to go: Click **Read** and follow the online prompts.

Basic Spectrum Analysis

Numerous applications can profit by a preliminary spectral screening. Here are instructions for setting up a basic spectrum protocol in Gen5.

It may be easier to follow these instructions if you have already watched the Gen5 Basic series of online tutorials: select Help>Tutorials, or if you've completed the learning exercises described in the Getting Started Guide.

1. Defining the reading Procedure

This assay example uses a kinetic read for analysis.

1. Select **File>New Protocol**
2. Open the **Procedure** (double click Procedure in the menu tree)
3. Click **Read** and change the **Read Type** to **Spectrum**
4. Set the Wavelength range: for this exercise set **Start** to **200** and **Stop** to **550**
5. Set the **Step** to **3**, and close the Read step
6. Click **OK** to save and close it.

2. Defining the Plate Layout

Define the plate layout in the usual way to reflect the arrangement of unknown samples, standards and blanks on the microplate.

3. Defining the Data Reduction Steps

Now that you've defined the reading parameters and plate layout, you can define the data reduction steps:

1. Select **Protocol> Data Reduction**
   Gen5 automatically sets up the a Well Analysis for Min/Max OD. If Blanks have been assigned to the plate, it will be preceded by and based on a blank-subtraction Transformation step.
2. Click **Well Analysis** to add another step
3. Enter a unique name for this step in the **Label** field
4. Select one of the offered **Calculation Type**s: Integral or Formula
5. Click **OK** to save and close the step
6. Click **OK** to save and close Data Reduction

4. Save the Protocol

1. Define the Reporting Requirements using the Report Builder or export options
2.  Save the protocol

Now you can run it in an experiment: select **File>New Experiment**

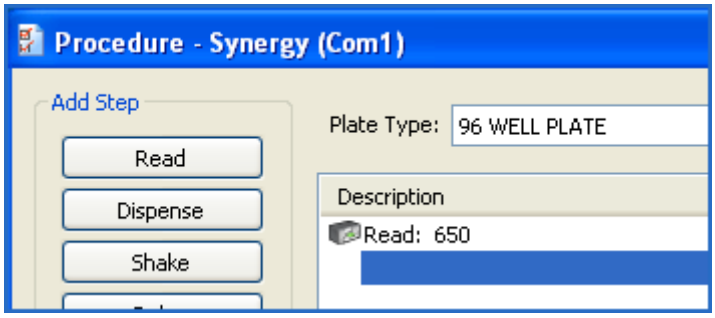
5. Viewing the Results

After you read the plate, you can take advantage of Gen5's Well Zoom to examine the results. This feature is described in the Kinetic Analysis chapter.

Protein Quantification: Endpoint Absorbance

Here are instructions for the Gen5 portion of running this type of assay — the easy part. Correctly mixing and dispensing the standards, and pipetting reagents to the plate is the tricky part. Follow the assay instructions closely and modify these steps, as needed. Click the links for instructions at each step.

1. Select **File>New Protocol**
2. Define the **Procedure**



   1. Select **Protocol>Procedure**
   2. Click **Read** to add one read step
   3. Keep the default settings:
      - Detection Method = **Absorbance**
      - Read Type = **Endpoint**
   4. Set the **Wavelength** using the drop-down or enter **650** in the nm field
   5. Click **OK** twice to close and save the **Read** step and the **Procedure**

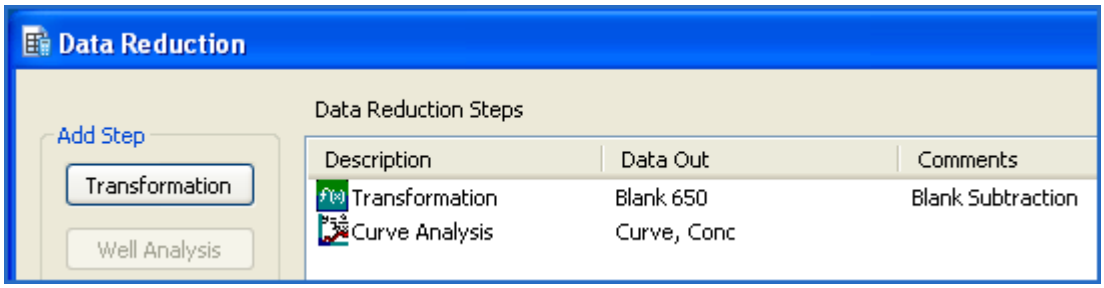
3. Define the **Plate Layout**

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 0	STD1 0	STD1 0	STD1 0	SPL1	SPL1	SPL1	SPL1	SPL9	SPL9	SPL9	SPL9
B	STD2 1	STD2 1	STD2 1	STD2 1	SPL2	SPL2	SPL2	SPL2	SPL10	SPL10	SPL10	SPL10
C	STD3 2.5	STD3 2.5	STD3 2.5	STD3 2.5	SPL3	SPL3	SPL3	SPL3	SPL11	SPL11	SPL11	SPL11
D	STD4 5	STD4 5	STD4 5	STD4 5	SPL4	SPL4	SPL4	SPL4	SPL12	SPL12	SPL12	SPL12
E	STD5 10	STD5 10	STD5 10	STD5 10	SPL5	SPL5	SPL5	SPL5	SPL13	SPL13	SPL13	SPL13
F	STD6 20	STD6 20	STD6 20	STD6 20	SPL6	SPL6	SPL6	SPL6	SPL14	SPL14	SPL14	SPL14
G	BLK	BLK	BLK	BLK	SPL7	SPL7	SPL7	SPL7	SPL15	SPL15	SPL15	SPL15
H					SPL8	SPL8	SPL8	SPL8	SPL16	SPL16	SPL16	SPL16

Set up Gen5's plate layout to match your placement of samples and standards on the plate, for example:

1.  Select **Protocol> Plate Layout**
2. In the Well Settings box, select the **Type** of specimen, first **Standards**, then **Blanks**, then **Samples**
3.  Define the **Concentration** of the Standards:
4. Set the **Replicates** to 4
5. Assign the well IDs to their corresponding locations in the plate matrix by clicking in the respective wells in the matrix. Use the **Auto Select** options to speed up your work.

4. Define the **Data Reduction**:



Gen5 creates a Blank Subtraction data set when you put blanks on the plate, as defined above. Click in the white space below the Transformation step:

- Click **Curve Analysis** to create a standard curve:
- **Data In**: **Well ID** is set to **STD**. Set the **Y Axis** = **Blank [650]**

- **Curve Fit**: Method is set to **Linear Regression**

5. Define the **Reporting Requirements**

Save the Protocol

Select **File> Save** when you're finished setting up the protocol. You'll be able to use this protocol repeatedly to run this assay in an experiment.

Select **File> New Experiment** and select the protocol when you're ready to run it, i.e. reagents are reconstituted, the plate is prepared, etc.