PLA 3.0

PLA 3.0

FROM ASSAY SETUP TO INSIGHTS

Quantitative response assays

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CONTENTS

1 QUANTITATIVE RESPONSE ASSAYS

Use Quantitative response assay documents to calculate the potencies of test samples relative to an established standard. You can also use them to determine absolute potencies based on stock solutions or raw materials.

What these documents do

Quantitative response assay documents cover all biological assay types that are based on quantitative response, that is, parallel-line, parallel logistic (full curve fits), and slope ratio assays.

They are often the first step in broader assay workflows, allowing you to structure and evaluate your data step by step, from defining samples and dilutions to assigning plate layouts and performing calculations. You can use their results as input for combination calculations or include them in automated workflows using protocols.

What you will learn

Performing daily assay runs is a common scenario in Good Manufacturing Practice (GMP) environments. In this topic you will learn how to set up the assay template that provides the setup of the Quantitative response assay documents that PLA 3.0 generates during the process.

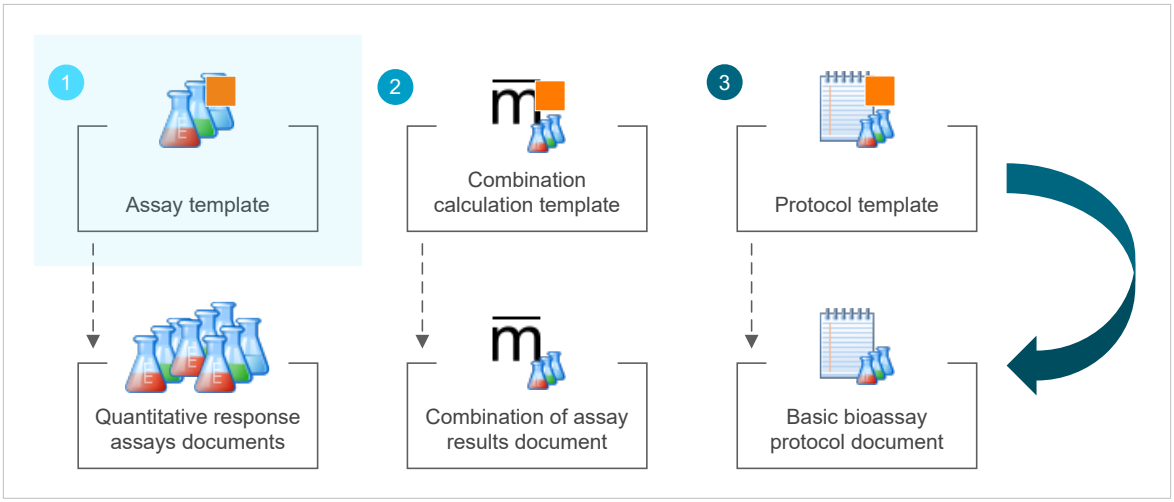


Figure 1-1 From plate to process step 1: Setting up an assay template

This topic belongs to a three-part series that guides you through the complete setup process in PLA 3.0. Each part focuses on one stage of this process. This topic forms the first part.

This series is based on the '40 Basic Bioassay Protocol' demonstration data included in version 27 of the Biological Assay Package. You will learn how to set up the data and understand the purpose behind each setup step.

What the setup looks like

For this use case, you work with an assay with a 96-well plate layout. The assay has one standard and one reference sample. The standard and test sample have one replicate each. All samples are diluted 12 times.

On the plate, you use wells A1 to A12 for the standard (reference) and wells B1 to B12 for the test sample. The rest of the wells remain empty.

The following figure shows the distribution of samples on the plate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
B	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
C	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
D	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
E	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
F	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
G	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
H	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty

Figure 1-2 Distribution of samples on a 96-well plate for a daily assay run

Note: The setup is kept simple and designed to introduce core concepts. It is not intended as a recommendation for practical use.

What you do step by step

The following table shows the tasks you need to perform to set up a Quantitative response assay document for this use case:

Step		Description	Task
1	**Document creation**	Create a new Quantitative response assay document.	Create the assay document
2	**Assay setup**	Set up the key components of your assay, that is, the standard and test sample assay elements and preparation schemes to define the doses and dimensions of your treatments.	Set up the assay elements Set up and assign the preparation schemes Define the plate size
3	**Assay analysis**	Configure the statistical model and tests used to evaluate your data.	Define the analytical settings Create the suitability tests

Step		Description	Task
4	**Samples and response values**	Arrange samples and dilution steps on a microtiter plate using position factors, enter observation data, and link it to your layout.	Define the assay layout Enter the observation data
5	**Calculation**	Run the analysis and review the reportable values.	Calculate the assay document
6	**Template creation**	Create a template out of the Quantitative response assay document you just set up.	Create a template from your document

Tip: Find more details on the Quantitative response assay document type in our Knowledge Center at https://help.bioassay.de/add_ons/concepts/quantitative_response_assays.html.

1.1 Create the assay document

Assay documents hold your assay data. Create the Quantitative response assay document required for this use case.

Procedure

To create the document:

1. In the **Navigator**, select the folder in which you want to create the document.
2. From the context menu, select **New**.
3. In the **Create a new document** dialog, on the **Available document types** tab, go to **Biological Assay Package** > **Quantitative response assay**.
4. You can create assay documents from scratch or using a template. For this use case, you create a document from scratch. Select 'New Quantitative response assay.'
5. To confirm your selection and create the document, select **Create**.

   Result: The **Content** editor opens, displaying your new document.
6. As the document name, enter `Daily Assay for Product X`.
7. Save the document.

What to do next

Set up the assay elements.

1.2 Set up the assay elements

Assay elements are the building blocks of your assay. They are the starting point for the setup of your analysis workflow, as they represent the individual samples you want to evaluate in your analysis. Set up the assay elements required for this use case.

Before you begin

You have created the assay document.

About this task

Each assay element plays a specific role in setting up your Quantitative response assay document, serving as a standard, test, or control sample, or a control line. By organizing samples as assay elements, you create a clear structure that supports the entire setup and analysis process.

You can tailor the analysis to reflect the purpose and characteristics of each sample by assigning key settings such as preparation schemes to each assay element. These settings determine how PLA 3.0 processes and evaluates each sample, supporting consistent and reproducible bioassay analysis.

Created from scratch, Quantitative response assay have one standard and one test sample. For this use case, you keep the number of assay elements as is.

Procedure

1. In the **Content** editor, expand **Setup** > **Assay elements**.

To set up the standard sample:

2. Expand the **Standard sample** element.
3. Rename the sample to `Ref`.

To set up the test sample:

4. Expand the **Test sample** element.
5. Rename the sample to `S1`.
6. Save the document.

What to do next

Set up and assign the preparation schemes.

1.3 Set up and assign the preparation schemes

Preparation schemes correspond to industry-standard practices of dose preparation protocols. They let you efficiently assign dilution sequences and replicate settings to assay elements, ensuring consistency and reducing redundant input during setup. Set up and assign the preparation schemes required for this use case.

Before you begin

You have set up the assay elements.

About this task

When setting up biological assays, consistent and accurate sample preparation is essential. To support you during this process, PLA 3.0 provides preparation schemes, centralized configurations that let you assign dilution sequences and replicate settings to assay elements, streamlining the setup process and ensuring reproducibility.

Created from scratch, Quantitative response assay have one preparation scheme, assigned to all assay elements. In this use case, the absolute potency of the standard and the test sample differ. You therefore add a second preparation scheme for the test sample and adjust the default preparation scheme setup as follows:

Setting	Default	Use case
Absolute potency	Not defined	Defined by stock solution
Dilution sequence	Geometric sequence	User-defined sequence

Procedure

To add a second preparation scheme:

1. In the **Content** editor, go to **Setup** > **Preparation schemes**.
2. In the **Creatable elements** pane, double-click **Preparation scheme**.

   Result: Your assay now has two preparation schemes.

To set up the first preparation scheme:

3. Expand the first **Preparation scheme** element.
4. Define the absolute potency:
   a. Switch from **Absolute potency: Not defined** to **Absolute potency: Defined by stock solution**.
   b. As the **Assigned/assumed potency**, enter `100`.
   c. As the **Assigned/assumed units**, enter `IU/ml`.
5. Set up the dilution sequence.
   a. Switch from **Geometric sequence** to **Defined sequence**.
   b. By default, defined sequences have one dilution step. This use case uses 12 steps. You therefore have to add 11 extra steps.

i. In the **Creatable elements** pane, right-click **Dose value**, and then select **Create multiple elements** from the context menu.

ii. In the **Create multiple elements** dialog, change the value of **Number of elements to create** to `11`.

iii. To add the elements, select **OK**.

Result: Your sequence now has now 12 dilution steps.

c. Starting at the first **Dose value** element, enter the following values: `5`, `3.33`, `2.22`, `1.48`, `0.99`, `0.66`, `0.44`, `0.29`, `0.2`, `0.13`, `0.09`, and `0.06`.

> **Tip:** Press Enter to confirm a value and jump to the next element.

To set up the second preparation scheme:

6. Expand the second **Preparation scheme** element.
7. Define the absolute potency:
   a. In the list box, switch from **Absolute potency: Not defined** to **Absolute potency: Defined by stock solution**.
   b. As the **Assigned/assumed potency**, enter `200`.
   c. As the **Assigned/assumed units**, enter `IU/ml`.
8. To set up the dilution sequence, repeat step 5 for the second scheme.

To assign the second preparation schemes to the test element:

9. Expand **Setup** > **Assay elements** > **Test sample**.
10. From the **Preparation scheme** drop-down list, select the second preparation scheme.
11. Save the document.

What to do next

Define the plate size.

1.4 Define the plate size

PLA 3.0 can handle a wide range of plate layouts and is not restricted to any particular format. Position factors in PLA 3.0 support you in defining plate layouts. They let you associate your observation data in PLA 3.0 with its physical location on the measurement system, such as a plate or rack.

Before you begin

You have set up and assigned the preparation schemes.

About this task

Quantitative response assay documents created from scratch do not contain position factors. You can define up to three position factors, intended for row, column, and plate.

Note: PLA 3.0 requires the plate position only if you set up multiple plates. As this use case uses one plate only, you only need the row and column positions.

Procedure

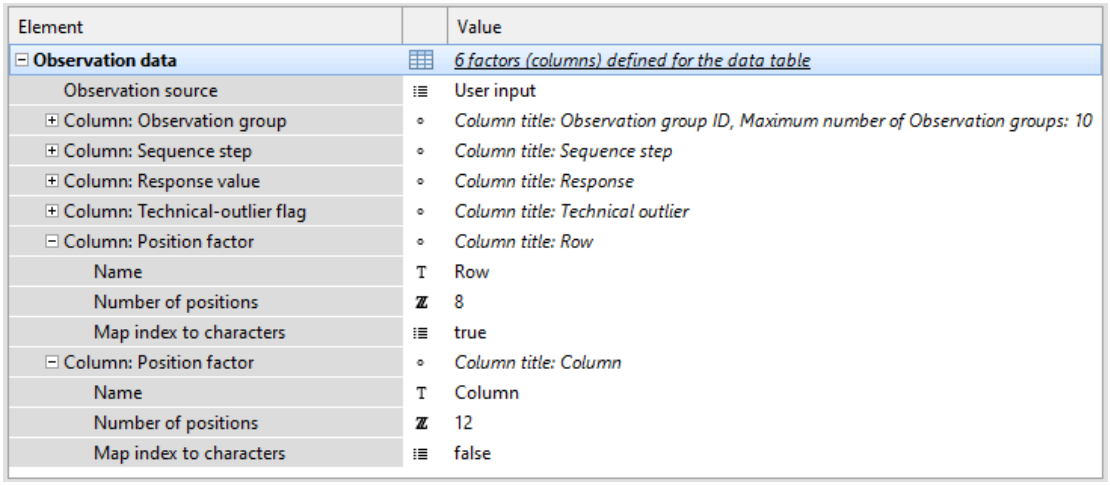
To define the size of your plate:

1. In the **Content** editor, go to **Setup** > **Observation data**.
2. In the **Creatable elements** pane, double-click **Column: Position**.

   Result: Your assay document now has one position factor.
3. To add the second factor, right-click the **Column: Position** element, and then select **Duplicate element**.

   Result: Your assay document now has two position factors.
4. Configure the rows of your plate.
   a. Rename the first position factor to `Row`.
   This is the column name shown in the **Observations** editor.
   b. Use the **Number of positions** setting to set the number of rows to `8`.
   c. By default, PLA 3.0 uses numbers for the rows in the **By Position** editor. To switch to alphabetical row labels ('A' to 'H'), select 'true' from the **Map index to characters** drop-down list.
5. Configure the columns of your plate.
   a. Rename the second position factor to `Column`.
   This is the column name shown in the **Observations** editor.
   b. Use the **Number of positions** setting to set the number of columns to `12`.

   Result: Your **Observation data** node should now look like this:



Element		Value
Observation data		*6 factors (columns) defined for the data table*
Observation source		User input
Column: Observation group		*Column title: Observation group ID, Maximum number of Observation groups: 10*
Column: Sequence step		*Column title: Sequence step*
Column: Response value		*Column title: Response*
Column: Technical-outlier flag		*Column title: Technical outlier*
Column: Position factor		*Column title: Row*
Name		Row
Number of positions		8
Map index to characters		true
Column: Position factor		*Column title: Column*
Name		Column
Number of positions		12
Map index to characters		false

Figure 1-3 Row and column position factors of a 96-well plate, displayed in the **Observation data** element of the **Content** editor

6. Save the document.

What to do next

Define the analytical settings.

1.5 Define the analytical settings

Use regression models to set up regression analysis for your assay elements. Selecting the appropriate regression model is an important step in defining your assay.

Before you begin

You have defined the plate size.

Procedure

To define the analytical settings:

1. In the **Content** editor, expand **Analysis**.
2. Expand **Analytical model**.
3. Switch from **Model: Linear parallel-line** to **Model: 4-parameter logistic fit**
4. Expand **Advanced settings**
5. As the **ANOVA model**, select **Residual-error ANOVA**.
6. Save the document.

What to do next

Create the suitability tests.

1.6 Create the suitability tests

Suitability tests are essential for verifying the integrity of both the overall assay and individual samples. They let you prove the validity of your calculation. Create the suitability tests required for this use case.

Before you begin

You have defined the analytical settings.

About this task

Suitability tests support you in ensuring that your analysis produces valid and reliable results. They define criteria that the data have to meet for the calculated outcomes to be considered scientifically sound and trustworthy.

Created from scratch, Quantitative response assay documents do not contain suitability tests. In this use case, you create an F-test on the significance of regression and one on the significance of non-parallelism, and use these tests as sample suitability tests.

Procedure

To create sample suitability tests:

1. In the **Content** editor, go to **Analysis** > **Suitability tests** > **Sample suitability tests**.
2. In the **Creatable elements** pane, double-click **F-test (hypothesis)**.

   Result: Your assay document now has one F-test for the significance of non-parallelism (default setting).
3. To add the second test, right-click the **F-test (hypothesis)** element, and then select **Duplicate element**.

   Result: Your assay document now has two F-tests.
4. Expand the first **F-test (hypothesis)** element.
5. From the **Type** drop-down list, select **Significance of regression**.

   Leave the other settings as is.
6. Save the document.

1.7 Define the assay layout

Define how your samples are arranged on your plate for this use case.

Before you begin

You have created the suitability tests.

About this task

Defining plate layouts involves two steps. First, you assign assay elements to wells. Next, you assign dilutions or sequence steps to wells. To perform these assignments, you use the primary display factor drop-down list ( ) in the toolbar of the **By Position** editor.

> **Tip:** To improve the readability of your table, you can activate the grid color view option. This use case uses the **By sequence step** grid coloring.

Procedure

To define the assay layout:

1. In the **By Position** editor, select **Observation group ID** as the primary display factor.
2. Assign samples to wells according to the following logic:

Cells	Observation Group ID
A1 to A12	[1] Standard sample: Ref
B1 to B12	[2] Test sample: S1
C1 to H12	Do not make any assignments.

Result: Your plate layout should now look like this:

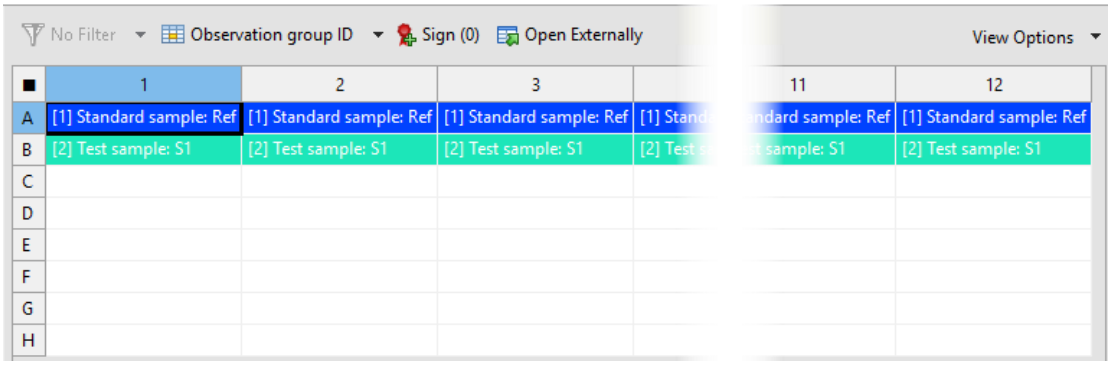


Figure 1-4 Assignment of assay elements to wells in the **By Position** editor, using the **Observation group ID** primary display factor (cropped)

3. Select **Sequence step** as the primary display factor and assign dilutions to sequence steps according to the following logic:

Cells	Sequence step
A1 and B1	[1] 5
A2 and B2	[2] 3.33
A3 and B3	[3] 2.22

Cells	Sequence step
A4 and B4	[4] 1.48
A5 and B5	[5] 0.99
A6 and B6	[6] 0.66
A7 and B7	[7] 0.44
A8 and B8	[8] 0.29
A9 and B9	[9] 0.2
A10 and B10	[10] 0.13
A11 and B11	[11] 0.09
A12 and B12	[12] 0.06
C1 to H12	Do not make any assignments.

Result: Your plate layout should now look like this:

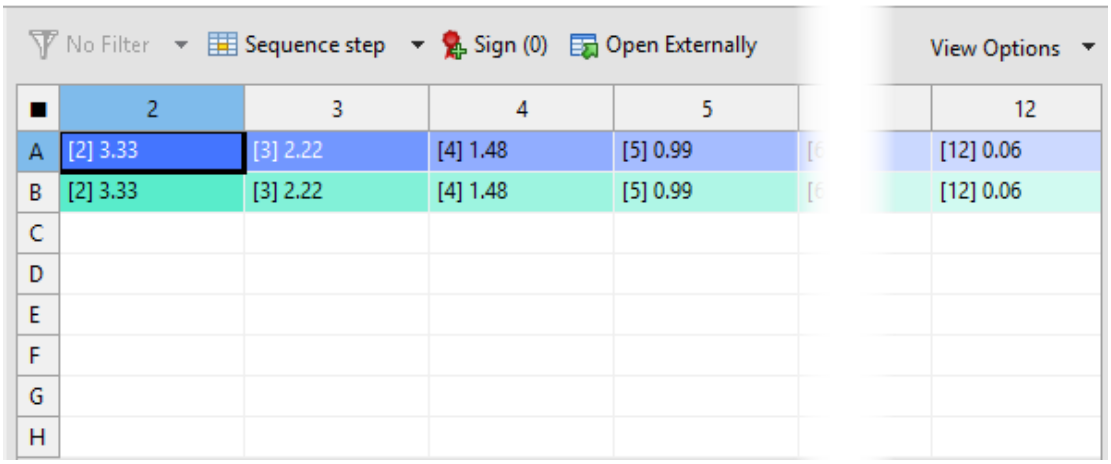


Figure 1-5 Assignment of dilutions to sequence steps in the **By Position** editor, using the **Sequence step** primary display factor (cropped)

4. Save the document.

What to do next

Enter the observation data.

1.8 Enter the observation data

Observation data refers to the original and unprocessed data that you collect or generate during the operation of your analytical methods. Add the observation data required for this use case.

Before you begin

You have defined your plate layout.

About this task

In this use case, you manually add your data by copy and paste. For your daily work, we recommend using one of the Data Acquisition Modules available for PLA 3.0.

> **Tip:** To improve the readability of your table, you can activate the grid color view option. This use case uses the **By sequence step** grid coloring.

Procedure

To enter the observation data:

1. In the **By Position** editor, select **Response** as the primary display factor.
2. Copy and paste the following values:

   Result:

	1	2	3	4	5	6	7	8	9	10	11	12
A	2	3	4	6	10	20	30	31	32	33	33.5	34
B	1.9	3.4	3.9	5	9	23	30	31	32	32.5	33	34

   Your observation data should now look like this:

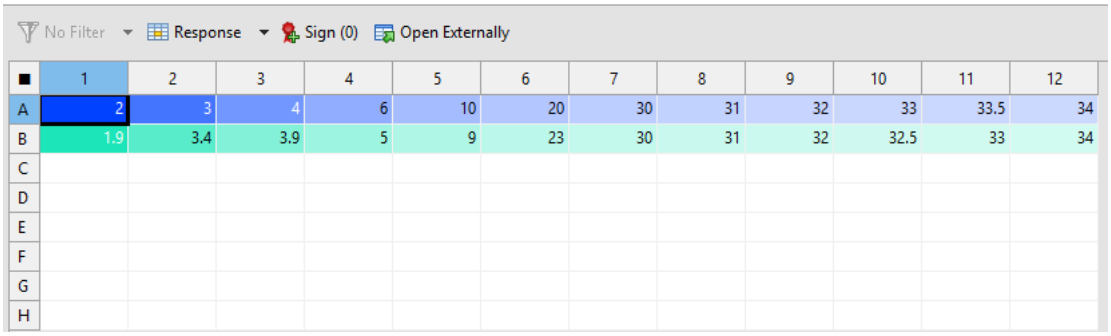


Figure 1-6 Observation data entry in the **By Position** editor, using the **Response** primary display factor

What to do next

Calculate the assay document.

1.9 Calculate the assay document

After you entered the observation data and finished the setup of your document, you can calculate the reportable values for this use case.

Note: Depending on the complexity of the document setup, the calculation time can vary from seconds to several minutes.

Before you begin

You have entered your observation data.

Procedure

To calculate your assay document:

1. On the action bar, select **Calculate**.

   Result: The **Calculation** dialog shows calculation details and informs you about the progress. PLA 3.0 displays the calculation result in the yellow status bar at the bottom of the dialog.

2. Select **Close**.

What to do next

Create a template from your document.

1.10 Create a template from your document

Create a template from the Quantitative response assay you created for this use case.

About this task

Note: You can use templates to restrict access to elements and define initial values. PLA 3.0 manages these restrictions through protection settings. For this use case, you will not use these settings.

Procedure

To create a template from your document:

1. On the **File** menu, select **Save as template**.
2. In the **Save template** dialog, select a target folder to save the template and confirm with **OK**.

   Result: PLA 3.0 saves the new template and displays it in the document pane.

To remove your observations:

3. Go to the **Observations** editor.
4. To remove all observation data, select all data columns and then select **Remove**.
5. Save the template.