Valita™TITER HS Assay
Instructions for Use
For the quantitative measurement of IgG antibody
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1.1 INTENDED USE

The Valita™TITER HS assay is a fluorescence polarisation assay for the quantitative detection of IgG antibody in cell culture supernatant and cell culture media. The Valita™TITER HS assay is indicated as a research use aid for detection of IgG in liquid samples.

1.2 BIOLOGICAL PRINCIPLES OF THE Valita™TITER HS IgG Assay

The Valita™TITER HS IgG assay is a single-step fluorescence polarisation assay for the rapid, quantitative detection of IgG antibody. The functional range of this assay is from 6 to 0.5 mg per litre of IgG antibody.

The assay is based on the principle that small molecules rotate faster than larger molecules in solution, and that the rotation rate can be determined by fluorescence polarisation. When an antibody in a sample is incubated with a specific antigen for the antibody that is labeled with a fluorescent probe, the presence of antibody-antigen complex in the sample can be detected by means of fluorescence polarisation. A problem with this technique is that for detection of a given antibody, a specific antigen for that antibody is required. A further problem with this technique is that is not suitable for quantitative detection of antibody in a sample.

In the Valita™TITER HS assay, IgG antibody in the sample binds to fluorescently labeled generic antibody-binding protein which has been pre-coated to the assay well of the microtitre plate. This results in a change in the fluorescence polarisation in the assay well of the microtitre plate; exciting the sample with plane polarised light at a wavelength corresponding to an excitation wavelength of the fluorescent dye, and detecting light intensity emitted by the fluorescent dye at an appropriate emission wavelength both in two planes, one of which is parallel to the plane of the excitation plane and one of which is perpendicular to the plane of the excitation light. The degree to which the emission intensity moves from the excitation plane (i.e. vertical) to a perpendicular plane (i.e. horizontal) – i.e. the change in polarisation between excitation and emission light - is a function of the degree of rotation of the fluorescent dye. When the generic antibody binding protein is bound to antibody, the complex will rotate slower than the unbound generic antibody binding protein, resulting in an increase in polarisation of emitted light. The concentration of IgG antibody in the sample of interest is determined from a predetermined calibration curve generated from known standard samples and the change in polarisation of light emitted upon sampling.
2.1 KIT COMPONENTS

Valita™ MAb HS Plates 10 No. 96 Well Microtitre plates: FITC G’ labelled protein, coated microtitre plates.

Valita™ MAb Un-coated Plates 10 No. 96 Well Microtitre plates: uncoated.

Valita™ MAb HS Buffer 2 No. 100 ml bottle containing 75 milliliters of Acetate, BSA, NaCl and Sodium azide (0.5 g per L) buffer.

2.2 MATERIALS REQUIRED BUT NOT PROVIDED

The user also requires:

- Configured fluorescence polarisation plate reader with dedicated computer and operating software;
- Valita™ APP analysis software [provided by Valitacell™ Ltd];
- Cell growth media (NOTE: Always use fresh/unused cell growth media to dilute test samples and to prepare IgG Standard Curve concentration samples);
- IgG* standard (used for preparation of standard curve IgG concentration samples);
- Calibrated pipettes, multichannel pipettes, and pipette tips;
- Sterile reagent reservoirs;
- Standard laboratory PPE;
- Cleaning solutions for cleaning and decontamination of work surfaces, gloves and equipment;
- Non-shedding paper wipes or cotton wool for cleaning and equipment decontamination.

*Note: When constructing the standard curve, for most accurate results use an IgG type which is as homologous to the IgG being assayed as possible. For example: matching IgG from the species, the subtype, e.g. IgG1 and the light chain type, i.e. lambda or kappa.

2.3 PRODUCT USE

For research use only.

2.4 WARNINGS AND PRECAUTIONS

- Dispose of kit materials according to your site waste disposal procedures.
- The Valita™ MAb Buffer contains Sodium azide 0.05% (mass / volume); please follow your specific site waste disposal procedure for azide containing waste.
GENERAL INFORMATION

• Refer to material safety data sheet information for safe handling procedures of any of the kit components.
• Follow your site-approved procedures for cleaning and decontamination of work surfaces and other lab equipment.
• For decontamination of the plate reader, follow the procedures described in the user’s manual. Also, complete the required decontamination document according to the procedure described in the user’s manual.
• Any deviation from the protocol may result in sub optimal results.
• Do not use reagents or other kit materials beyond their expiration date.
• Do not pool reagents within a kit or between reagent kits.
• Prior to using for the first time, or following extended storage, the Valita™MAb Buffer requires mixing.
• To avoid contamination, wear clean gloves when handling Valita™MAb Plates and during reagent and sample additions.

2.5 STORAGE INSTRUCTIONS

• The Valita™TITER HS Kit must be stored at 2-8°C in an upright position.
• When stored and handled as directed, reagents are stable until the expiration date.

2.6 INDICATIONS OF REAGENT DETERIORATION

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration is necessary.
3.1 PREPARATION OF KIT COMPONENTS

Valita™MAb HS Plates and Valita™MAb Buffer come ready-to-use.

Prior to first use or following extended storage the Valita™MAb Buffer should be mixed. This can be accomplished by gentle inversion of the capped bottle 3 to 5 times. An automatic roller mixer may also be used.

Aliquot the required volume of Valita™MAb Buffer into a sterile multi-channel pipetting trough and allow the buffer to come to room temperature before use. After aliquoting, return the remaining buffer to refrigerated storage to avoid repeated warming and cooling of the buffer. Allow samples, standards, and cell growth media to come to room temperature before use.

Allow the Valita™MAb HS Plates to come to room temperature before opening the sealed foil pouch.

3.2 PREPARATION OF TEST AND CONCENTRATION STANDARD SAMPLES

Test Samples and IgG standard curve concentration standards must be diluted in cell growth media. Always use fresh/unused cell growth media to dilute test samples and to prepare the IgG standard curve concentration samples. Use the same cell growth media that was used during the growth of cells from which the test samples are taken. Different formulations of growth media may have different fluorescence profiles that can result in erroneous results if one formulation is used during cell growth and different cell growth media is used to dilute test samples or IgG standards.

All test samples must be diluted at least 1:1 in cell growth media before being tested.

We recommend that when assaying fed batch studies that samples are diluted at least 1:40 in fresh cell growth media. This is the ONLY dilution factor which you need to record and incorporate into Valita™APP to calculate your final sample concentrations - dilution caused by adding 60 μL of the (diluted) sample to 60 μL of buffer is already included in the software algorithm and thus DOES NOT have to be taken into account by the user.

Ensure diluted samples are thoroughly mixed before they are transferred to the Valita™MAb HS Plate wells.

3.3 GENERAL INFORMATION

Always place the Valita™MAb HS Plate on a clean flat surface before adding Valita™MAb Buffer or samples to the wells of the plate.
Carefully add samples to the wells of the Valita™MAb HS Plate wells to avoid cross-contamination between samples.

Never use a Valita™MAb HS Plate that has visible cracks or that is chipped.

Valita™Mab HS Plates are for single use only. After using the plates to test one set of standards and/or samples, dispose of the plate according to your site waste disposal procedure.

We do not recommend pipetting sample volumes <5 μL.

### 3.4 INSTRUMENT SETUP

Before performing the Valita™TITER HS assay, prepare the plate reader for use as described in the user’s manual. Turn on the plate reader and allow at least 30 minutes for the instrument to warm up.

Ensure the connection between the plate reader and dedicated computer are correct. Turn on the computer, and initiate the plate reader software, inputting additional experimental information into the software as required. The required additional information is determined by the testing requirements for each set of plates to be tested.

### 3.5 STANDARD CURVE

On occasion [different type of molecule*, QA procedure, etc.] a standard curve is required.

The individual user can determine the number and concentration of each individual standard in the standard curve. In general we recommend preparing a 6-point standard curve with concentrations as outlined in the table below.

For best results, each standard curve should be performed in triplicate.

Note: When constructing the standard curve, for most accurate results use an IgG type which is as homologous to the IgG being assayed as possible. For example: matching IgG from the species, the subtype, e.g. IgG1 and the light chain type, i.e. lambda or kappa.

Standard curve results may differ slightly between batches of Valita™MAb HS plates. Thus, preparing and testing of a new standard curve is recommended for each lot of Valita™MAb HS plates.
ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Dilution Number</th>
<th>Concentration (mg per litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>0.375</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

A simple way of calculating the dilution series is as follows:

\[
\frac{\text{Desired concentration of highest curve point [g/L]}}{\text{Concentration of standard available [g/L]}} \times \text{Desired volume of highest standard [µL]} \]

**Example:**
- Available standard in lab has concentration of 14.3 g/L.
- User wants the top point of their curve to be 6 mg/L.
- User wants to prepare 1ml of the highest concentration of standard to ensure enough sample for serial dilution.

\[
\frac{0.006 \text{ [g/L]}}{14.3 \text{ [g/L]}} \times 1,000 \text{ [µL]}
\]

= 0.419 µL of standard would be diluted in 994.4 µL diluent (i.e. fresh media) to obtain a final concentration of 6 mg/L.

*However, it is NOT advisable to pipette samples or standards < 5 µL. It would therefore recommended to first dilute the available standard. E.g: A standard at 14.3 g/L diluted 1:100 results in a concentration of 0.143 g/L. A workable volume of 41.9 µL would then be added to 958.1 µL diluent to make a concentration of 6 mg/L (see table below).

Use a clean pipette tip for each dilution.

The following table outlines a standard dilution preparation table for the given example standard calculation above. This is an **EXAMPLE only**. Each standard curve calculation will vary depending on the concentration of the standard available.
## ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>Volume to dilute (μL)</th>
<th>Diluent (μL)</th>
<th>Total volume (μL)</th>
<th>Starting conc. (g/L)</th>
<th>Final Conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41.9</td>
<td>958.1</td>
<td>1000</td>
<td>0.143</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>200</td>
<td>400</td>
<td>0.08</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>200</td>
<td>400</td>
<td>0.04</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>200</td>
<td>400</td>
<td>0.02</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>200</td>
<td>400</td>
<td>0.01</td>
<td>0.375</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>400</td>
<td>400</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

[Diagram of dilution process from 1 to 6]
4.1 PREPARATION AND READING OF A BACKGROUND BLANK PLATE

- Open the pouch containing the Valita™MAb Un-coated plates and place on a clean flat surface.
- Using a calibrated multichannel pipette and clean pipette tips add 60 μL of the Valita™MAb buffer.
- Using a multichannel pipette and clean pipette tips add 60 μL of samples or standards into the appropriate wells of the background plate, such that the plate layout matches exactly that of the corresponding Valita™TITER HS test plate.
- After adding the samples or standards, mix the liquid contents of each well. This is achieved by aspirating and re-dispensing approximately 100 μL within each well a total of 3 times per well. Use a clean pipette tip for each well to ensure that there is no cross-contamination between wells. Execute the aspirating and re-dispensing in a controlled manner to ensure that bubbles are not created in the wells.
- Load the plate into the plate reader and start the plate reading procedure.

*NOTE:* There is no 30 minute incubation required with the un-coated background blank plate.

4.2 Valita™TITER HS ASSAY PROCEDURE

1. Turn on plate reader and dedicated computer.

2. Bring kit components and samples to room temperature.
   - Aliquot the required volume of the Valita™MAb Buffer into a sterile multichannel pipette trough and allow the buffer to come to room temperature.
   - Open the pouch containing the Valita™MAb HS Plate and place the plate on a clean flat surface.

3. Enter experimental parameters into the software.

4. Review existing standard curve and prepare new standard curve if required.

5. Dilute test samples in growth media resulting in a final sample volume of at least 70μL. Neat samples should be diluted at least 1:1 in growth media to avoid matrix effects [we recommend that when assaying fed batch studies that samples are diluted at least 1:40 in fresh cell growth media].

<table>
<thead>
<tr>
<th>Example No.</th>
<th>Cell Culture Sample Volume</th>
<th>Media diluent</th>
<th>Dilution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40 μL</td>
<td>40 μL</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>30 μL</td>
<td>60 μL</td>
<td>1:2</td>
</tr>
<tr>
<td>3</td>
<td>20 μL</td>
<td>60 μL</td>
<td>1:3</td>
</tr>
<tr>
<td>4</td>
<td>16 μL</td>
<td>64 μL</td>
<td>1:4</td>
</tr>
<tr>
<td>…</td>
<td>…</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>…</td>
<td>12.5 μL</td>
<td>500 μL</td>
<td>1:40</td>
</tr>
</tbody>
</table>
ASSAY PROCEDURE

6. Working promptly, using an appropriately sized calibrated multichannel pipette (preferably a pipette that has a maximum dispense volume of 100 µL) and clean pipette tips add 60 µL of the Valita™MAb Buffer into each of the plate wells [Valita™MAb HS Plate and the Valita™MAb HS Un-coated plates] to be used*.

*NOTE: Complete the buffer addition step and subsequent steps without interruption and as quickly and efficiently as possible. Unnecessarily extending the time from buffer addition to final sample addition may result in suboptimal results, such as lower than expected signal values.

7. Working promptly, using an appropriately sized calibrated pipette (or multichannel pipette) and clean pipette tips add 60 µL of sample into individual wells of both the Valita™MAb plate and the Valita™MAb HS Un-coated plate blank background plate.

8. After completing the addition of the samples into all wells, using a multichannel pipette and clean pipette tips, mix the liquid contents of each well. This is achieved by aspirating and re-dispensing approximately 100 µL within each well a total of 3 times per well. Use a clean pipette tip for each well to ensure that there is no cross-contamination between wells. Execute the aspirating and re-dispensing in a controlled manner to ensure that bubbles are not created in the wells.

9. After mixing the liquid in all wells, place the plates in the dark, on the clean flat surface for 30 minutes. Plates may be covered at this time with plate covers to ensure that contamination from the surrounding environment does not occur.

10. Read on Plate Reader.
5.1 SOFTWARE OVERVIEW
Valitacell™ assays are configured for use on microtitre plate readers and thus require the plate reader software and the Valitacell™ analysis module [Valita™APP].

Plate reader software - The Plate reader software [e.g. PHERAstar software is required to operate the plate reader and the output [.csv file] from the plate reader is automatically moved to a dedicated results folder. Each application or test has its own PHERAstar protocol and users can ‘establish’ new protocols using the ‘Manage protocol function’. All Valitacell™ assays are pre-configured by Valitacell™ trained personnel and there is no requirement to alter any protocol settings.

Valita™APP - The Valita™APP software retrieves the Pherastar output [.csv file] and performs the data analysis. The Valita™APP is pre-installed and configured by Valitacell™ trained personnel.

5.2 DATA MANAGEMENT
It is entirely up to the user to decide their specific file naming structure. During installation Valitacell™ will implement the desired results path with each customer in accordance with their needs.
5.3 RUNNING AN ASSAY – PLATE READER

The following is for illustrative purposes and describes in detail the procedure for running a Valita™TITER HS assay on a Valitacell™ PHERAstar Plus plate reader.

1. Open the PHERAstar plus software by double clicking the ‘PHERAstar plus’ icon on the desktop.

2. Select the specific assay you are going to run e.g. Valita™TITER HS.

3. Select ‘Change layout’.
ANALYSIS

4. Double click on ‘Empty’ and then using the cursor, select all the wells on the displayed 96-well microtitre graphic. This will result in a clear 96-well graphic.
5. The next step is to tell the plate reader which wells you want to read. This is done by clicking on ‘Sample’ and then using the cursor, select the wells on the displayed 96-well microtitre graphic which correspond to the wells on the plate you wish to read [e.g. A1 to C12]. Click ‘Start measurement’.
6. The next step is to name this plate [it is important to note that the name provided here will be the file name used when the .csv file for this experiment is moved to the results folder] by inserting the file name you wish to use into the Plate Identification section under ID1 [e.g. PLATE NAME].

(*NOTE: For every reading of a Valita™Mab HS plate, a corresponding uncoated blank background plate containing Mab buffer and samples or standards is required. See section 4.1)*
5.4 ANALYSING THE DATA

1. Open the Valita™APP by double clicking the ‘Valita™APP’ icon on the desktop.

2. Select the specific assay you are going to run e.g. Valita™TITER HS.

3. Click on the ‘Choose curve’ tab on the LHS of the screen.

   a. Determine if your experiment requires a new standard curve or if you will be selecting a curve from the database.

   b. If your experimental design included a new standard curve then proceed with the following steps:

      • Select ‘Import New Curve’ and select the file within the results folder which contains the standard curve data using the ‘Upload CSV File’ button;
• The raw data will be displayed on the screen:

![CURVE RAW DATA](image)

![UPLOAD CURVE BLANK CSV FILE][image]

• Next, upload the background blank plate by selecting select ‘Upload curve blank CSV file’ and select the file within the results folder which contains the background blank data using the ‘Choose file’ button. The raw data will be displayed on the screen:
ANALYSIS

- Insert the concentrations of each well in accordance with the dilutions prepared for the standard curve.

<table>
<thead>
<tr>
<th>CONCENTRATIONS VALUES</th>
<th>Clear</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
<td></td>
</tr>
<tr>
<td>A 6 3 1.5 0.75 0.375 0</td>
<td></td>
</tr>
<tr>
<td>B 6 3 1.5 0.75 0.375 0</td>
<td></td>
</tr>
<tr>
<td>C 6 3 1.5 0.75 0.375 0</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
</tbody>
</table>

- The standard curve will automatically appear as a graphic in the curve plot area:

- Save this curve to the database with your selected file name and curve ID as you may chose to use this again (*Note: you must input a value under “Curve ID”, otherwise the file will not save):
• Choose the curve from the database.

c. If your experimental design does not require a new standard curve then simply select an existing standard curve from the database using the drop down menu provided.

4. The next step is to select the test results by selecting the ‘Upload Valita™TITER HS plate’ tab on the LHS of the screen.
First, select the test results by selecting ‘Upload probe plate CSV files’.

The next step is to select the background blank plate for the test samples by selecting the ‘Upload blank plate CSV file’. The raw data will be displayed on the screen.

5. Insert the corresponding dilutions manually or upload a pre-determined dilution file and the raw results will be displayed in a tabular format.
6. The next step is to view the test results by selecting the ‘View results’ tab on the LHS of the screen. The results can by downloaded in summary format as a .csv file or a summary PDF report can also be downloaded.

7. The final optional step is to save these results to a database and to insert some brief meta data relating to that specific experiment by selecting the ‘Save results to DB’ tab on the LHS of the screen.
5.5 MAINTENANCE & SUPPORT

Teamviewer is pre-installed on the Valitacell™ laptop and we can troubleshoot most queries remotely. Just log a call at {HYPERLINK "mailto:support@valitacell.com"} and we will schedule a troubleshooting session with one of our trained personnel.

5.6 LIABILITY

**Product Use:** For Research Use Only.

The Valita™TITER HS Kit is only intended for the determination of IgG concentration in liquid samples. Use of the Valita™TITER HS assay outside the IgG concentration range of 0.5 to 6 mg per litre is not valid.