High throughput Fc- and Fab-specific titer assays using capture beads and fluorescence detection.

Application Note

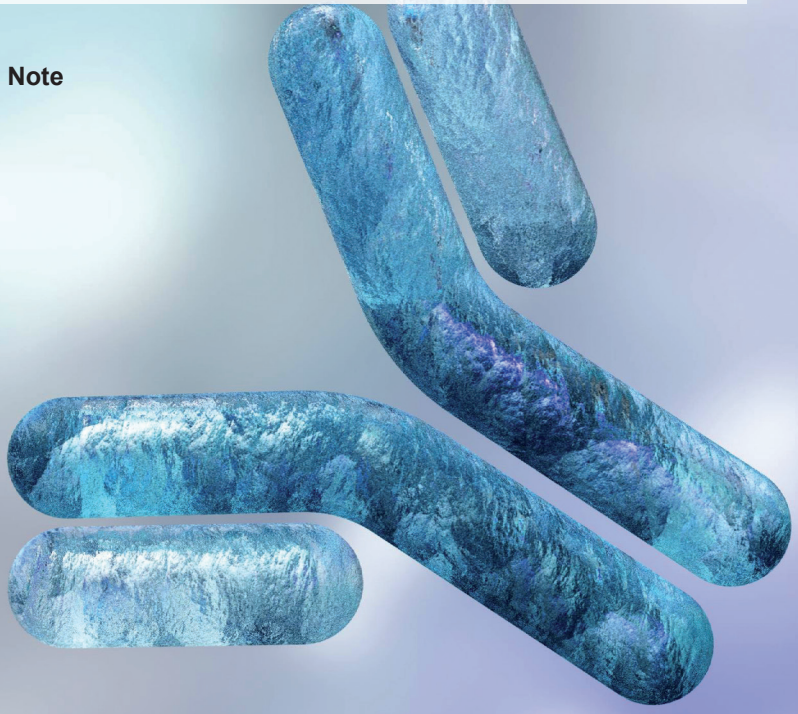

PLATE BASED TITER QUANTIFICATION WITH PAIA TITER ASSAY AND TECAN SPARK® MULTIMODE READER

INTRODUCTION

Generating and analyzing a large number of culture samples for titers and other quality-related parameters is a complex process that requires effective tools to achieve high throughput. The biopharmaceutical industry is heavily trending towards automation and miniaturization of these processes by favoring assays with simple workflows that only require small sample volumes.

The PAIA assay is a fast, cost-effective, and automatable technology that addresses this challenge. This completely plate-based assay enables monitoring of a range of parameters, including titers for Fc-containing molecules such as mAbs, biospecifics, fusion proteins and Fab fragments, to serve various applications in the bioprocess development area.

The Tecan Spark multimode reader is a versatile, high-end detection instrument that offers conventional functions such as absorbance, fluorescence, luminescence and image-based analysis in a modular and upgradeable design, for effortless handling of routine and complex assays for a range of biochemistry and cell biology applications. A highlight is the innovative fluorescence Fusion Optics, which combines the flexibility of monochromators with the sensitivity of filter-based detection within a single instrument. This set-up ensures a high degree of versatility for assay development and optimization, combined with exceptional sensitivity for fluorescence-based applications, even in the low signal range.

This application note describes how cell culture labs can implement the PAIA titer assays – PA-104 for Fc and PA-105 for Fab – on the Spark reader.

ASSAY TECHNOLOGY AND WORKFLOW

PA-104 and PA-105 are competitive bead-based assays containing similar components: capture beads carrying an antibody and a fluorescently labeled reagent – Protein A and an anti-human Fab-specific ligand in the Fc and Fab titer assays respectively – that specifically binds to the analyte in the sample.

The reagent binds to either the antibody on the capture beads or the analyte in the sample. If it binds to the analyte, it remains in solution and produces a fluorescent signal; the higher the fluorescence intensity, the higher the analyte concentration in a sample.

PAIA assays use special 384-well microplates in which the whole assay takes place. Each well in the microplate contains a transparent pyramid in an otherwise black well, a feature that allows for the separation of the capture beads from the solution. The fluorescence intensity of the solution can then be measured to directly determine the amount of analyte in the sample. The generic workflow is shown in Figure 1.

Microplates loaded with dried capture beads (1) are reconstituted by the addition of reagent solution and sample (2). The plate is then transferred to an orbital shaker and mixed for 15 minutes (3), followed by a minimum five-minute waiting time to allow the beads to settle (4). The plate is then ready for measurement (5). A complete plate can be measured in three minutes or less.

PAIA assays are very robust and therefore well-suited for automation. Analytes are detected by standard fluorescence measurement, a technique that is much less matrix sensitive – for example, to sample viscosity – than alternative methods such as fluorescence polarization or FRET.

The reagents can be handled in daylight as they are not light-sensitive, and the automated workflow can be paused at any point in time if desired, for example, to allow several assay plates to be shaken subsequently and then read in one go on the Spark. Sample preparation is straightforward as the assay tolerates cells, eliminating the need for a cell removal step. The assay kits can also be partially used and stored for up to three months.

Figure 1: The PAIA assay workflow.

The Spark reader is especially suitable for the PA-104 and PA-105 assays. The high performance PMT used for fluorescence supports a spectral range up to 900 nm and delivers exceptional sensitivity in the red wavelength area, which is known to have very little background fluorescence from cell culture supernatants. The z-focus in bottom read mode allows automatic determination of the best z-parameter for the PAIA plates. The selectable smooth plate transport allows plate insertion into the reader with minimal disturbance of the sedimented beads prior to the measurement.

The assay parameters are summarized in Table 1.

Assay parameters	PA-104	PA-105
Specificity	Fc (Protein A reagent)	Human Fab
Sample preparation	No cell removal necessary, dilution if required	No cell removal necessary, dilution if required
Assay range	2-400 µg/ml	1-70 µg/ml
Total assay volume	40 µl	60 µl
Sample volume	5-20 µl	5-20 µl
Limit of detection	2 µg/ml	1 µg/ml
Assay time	20 minutes for up to 384 samples	20 minutes for up to 384 samples
Read time on the Spark	3 minutes	3 minutes

Table 1: General parameters for the PA-104 and PA-105 assays.

MATERIALS AND METHODS

- PA-104 Fc titer assay kit
- PA-105 Human Fab titer assay kit
- Trastuzumab (human IgG1)
- Fab fragment standard
- Tecan Spark multimode reader
- BioShake XP orbital shaker (QInstruments)

The assays were performed according to their respective standard protocols. For the PA-104 assay, 35 µl of reagent was mixed with 5 µl of sample, and for the PA-105 assay, 54 µl of reagent was mixed with 5 µl of sample. Both assay plates were shaken on the BioShake for 15 minutes and beads were allowed to settle for 5 minutes. The shaking speeds were 2,300 rpm and 1,800 rpm for PA-104 and PA-105 respectively.

Eight calibration standards were generated by serial dilution of stock solutions of trastuzumab and the Fab fragment standard and measured in triplicate using the parameters listed in Table 2.

Measurement	Monochromator (or filter optics)
Measurement mode	Fluorescence bottom read
Excitation	630 nm (15 nm bandwidth)
Emission	665 nm (15 nm bandwidth)
Gain	Optimal
Z-position	Manual
Number of flashes	30
Lag Time	0 µs
Integration time	40 µs
Plate layout	GRE384fb
Multiple reads per well (square)	1x1
Multiple reads per well (border)	500 µm
Plate transport	Smooth mode

Table 2: Spark reader settings for the PAIA titer assays.

RESULTS

A z-scan for fluorescence bottom reading – a special feature of the Spark – was used to determine the optimal z-position for measuring the PAIA plates (Figure 2). A clear maximum fluorescence intensity was identified, and this z-position was used for all subsequent measurements. This optimization parameter is valid for all PAIA assays and can be saved in the Tecan method file.

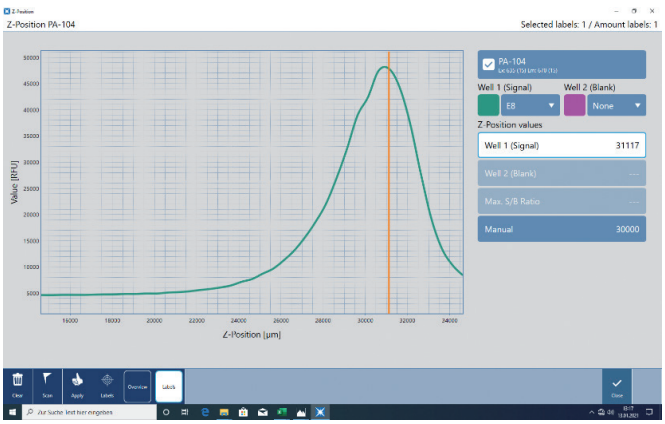

Figure 2: Screenshot of a Spark reader z-scan on a PAIA plate.

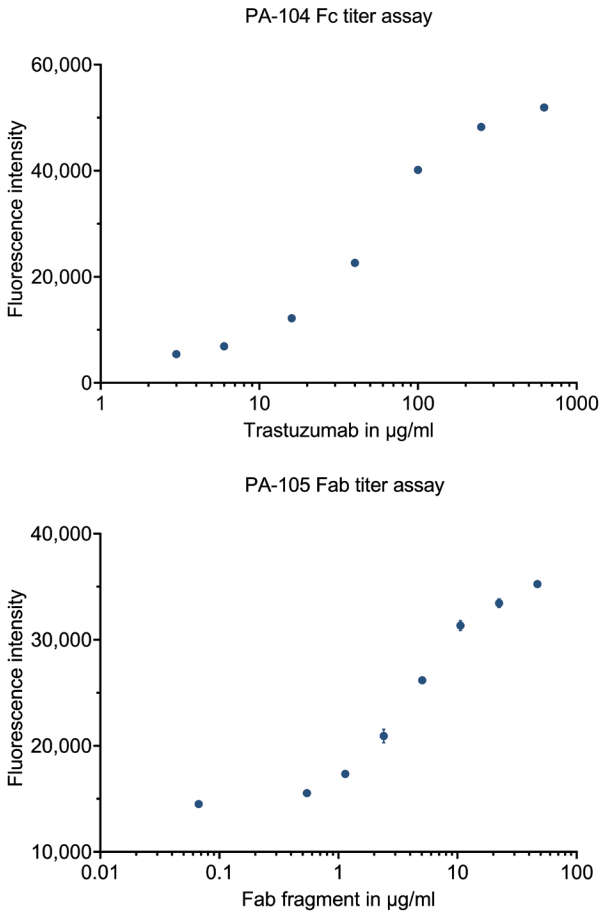

Figure 3: Calibration curves for the PA-104 and PA-105 assays.

SparkControl™ Magellan™ software was used to generate the calibration curves (5-parameter fit) and calculate unknowns directly on the Spark reader. Alternatively, the standalone PAIA analysis software can be used. Calibration curves obtained with the two assays (Figure 3) showed triplicates with CVs of less than 3 %, demonstrating the stability of the readout on the Spark reader.

CONCLUSIONS

This application note demonstrates that the Spark multimode reader is well-suited to PAIA titer assays for Fc-containing molecules (PA-104) and Fab fragments (PA-105), enabling very fast screening of hundreds of cell culture samples. Set-up and optimization of the method (z-scan) takes a matter of minutes and yields robust read-outs with low CVs. The Spark's high performance PMT – supporting a spectral range up to 900 nm – combined with z-scanning in bottom mode and a smooth mode of plate transport make it the ideal reader for the PAIA titer assays.

About the authors

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