Rapid PAIA Titer assays for antibodies and Fc Fusion proteins on the CELLAVISTA® and NYONE® Imagers

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Application Note
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Abstract

The use of the PAIA Fc Titer assay PA-104 on CELLAVISTA® or NYONE® imagers enables the super-fast titer analysis of IgGs in small volumes of cell culture supernatant.

The equivalent of four 96-well plates can be analyzed in 25 minutes in a 384-well plate without sample preparation and a workflow which is easily automatable.

This combination of SYNENTEC´s imagers and the patented PAIA technology provides an efficient way to ensure monoclonality and to monitor cell growth, viability and productivity on just one instrument.

KEYWORDS: CLD; IGG; PAIA TITER ASSAY; 96-well and 384-well plates; MONOCLONALITY; FC FUSION PROTEINS; MAB; CHO CELLS; FED-BATCH TITERS; 96-DWP CULTURES

Introduction

Hundreds to thousands of clones have to be screened during the cell line development process to find the well growing clones with high productivity. These processes are usually performed in 96-well and 384-well plates. Thus, there is limited sample volume available for analysis and it is desirable to get the results as quickly as possible.

Running PAIA assays on CELLAVISTA® or NYONE® imagers presents a solution that is easily automatable even with rather affordable liquid handlers, saves time and lab space and eliminates the need to invest into additional expensive instruments like the Octet (ForteBio).

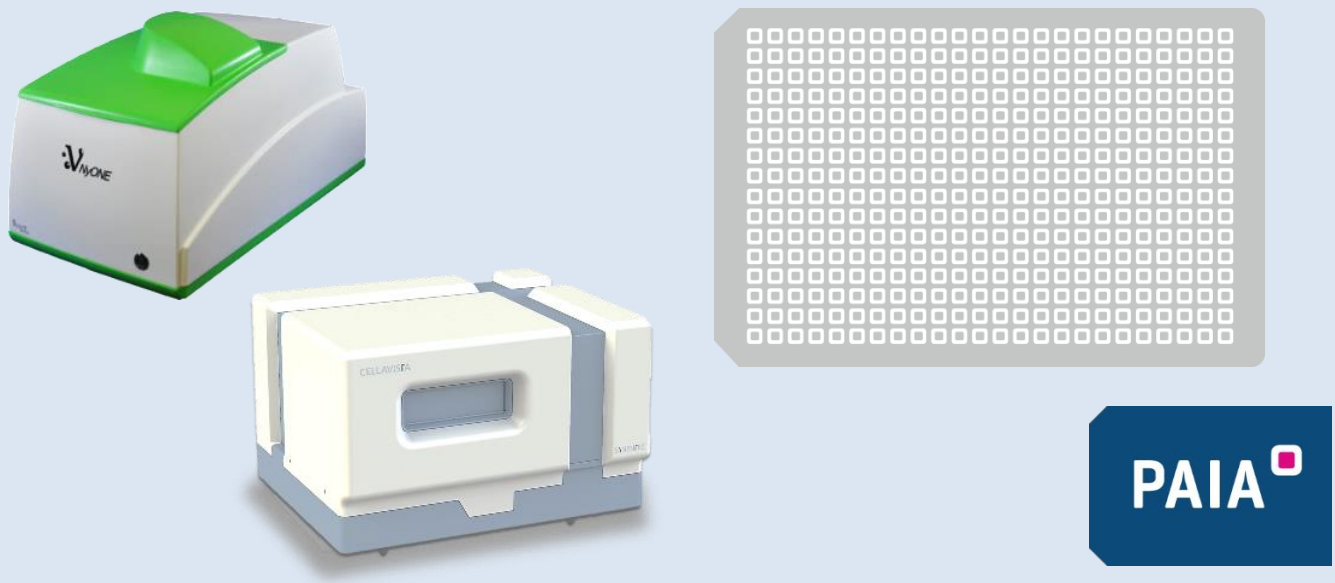

Fig. 1: CELLAVISTA® and NYONE® imagers and the schematic representation of a PAIA*plate*

Applications

PA-104 Fc titer assays are used to identify high producer clones in static 96- or 384-well plate cultures, typically 14 days after single cell deposition. Clone rankings can be obtained without using calibration standards or by using already existing calibration data from previous assays.

Fed-Batch titers from e.g. 96-DWP cultures can be measured by diluting samples with fresh medium depending on the expected titers.

Both applications have been automated with liquid handlers equipped with 96-channel heads, e.g. from Hamilton, Tecan, Agilent and Dynamic Devices.

Assay Principle

PAIA assays are fluorescent bead-based immunoassays that are carried out in single-use 384-well plates. Each of the wells has a transparent protrusion on an otherwise black well bottom. The protrusions are used to separate bead-bound marker from unbound fluorescence marker.

The Fc titer assay kit (PA-104) is designed for the rapid quantification of all Fc-domain containing proteins like IgGs, bispecifics and Fc Fusion proteins in sample volumes of 5 - 15 µL. The assay uses capture beads with an immobilized IgG and a fluorescence labeled Protein A reagent as the marker.

The assay is a competitive assay in which the analyte in the sample binds to the labeled ProteinA and any remaining Protein A reagent is bound to the beads, which leads to a decrease of fluorescence intensity. Hence, high analyte concentrations yield high fluorescence intensities and low concentrations low fluorescence intensities.

The dynamic range starts from a few µg/mL and extends to 400 µg/mL. Samples can be taken directly from cell culture supernatants and be used in the assay without dilution.

Cells do not have to be removed from the samples.

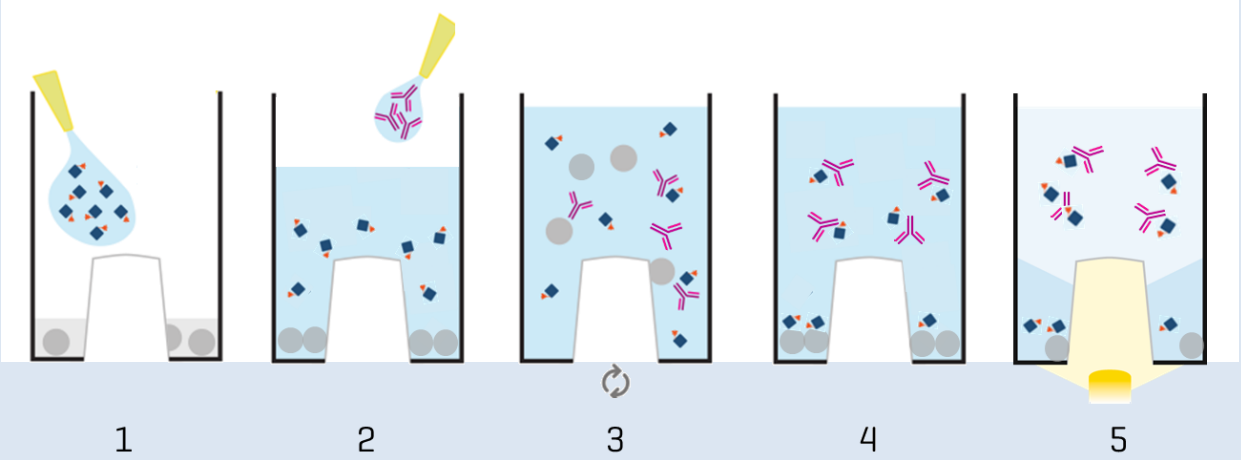

Fig. 2: PAIA assay workflow

1 - Addition of ProteinA reagent, 2 - Addition of sample, 3 - Shaking on an orbital shaker for 15 mins, 4 - Bead settling 5 mins and 5 - Read out on Cellvista or NyONE imager.

Protocol

The Fc titer assay was performed according to the standard protocol. Firstly, ProteinA reagent was added to the wells of the PAIAplate containing dried capture beads. Afterwards the samples were added. The IgG1 was tested at different reagent:sample volume ratios to illustrate how the assay range can be modulated. The comparison of the three analytes was performed with volumes of 30 µL ProteinA reagent and 10 µL sample.

Calibration standards for an IgG1, an IgG4 and a Fc fusion protein were prepared in GIBCO® FreeStyle™ CHO Expression medium. Calibration standards were measured in triplicates.

The PAIAplates were incubated for 15 minutes on an orbital shaker at 2300 rpm. After 5 minutes of bead sedimentation the measurement was performed with the 10x objective at an excitation wavelength of around 640 nm (red channel on the NYONE®).

Using the NYONE® wizard for PAIA assays, only one image in the center of each well was taken at a fixed focus position, resulting in a measurement time of 3 minutes for the whole plate.

The measured values for the fluorescence intensity were imported into the PAIA evaluation software for data analysis.

Results

The calibration curves for all three analytes (Fig.3) show very similar shapes with only slight differences.

The titers are most accurate in the concentration range that corresponds to the steep part of the calibration curve. The assay range can be easily tuned by the reagent:sample volume ratio, which is only a small change in the protocol (Fig. 4).

If higher sensitivity is needed because the expected IgG titers are low, one may use a reagent:sample volume ratio of 25:15 µl which will shift the assay range towards lower concentrations and allow reliable analysis of samples below 10 µg/mL.

On the contrary, if the titers in the samples are expected to be on the higher end, a 35:5 µL reagent:sample volume ratio is recommended, allowing to quantify non-diluted samples up to 400 µg/mL.

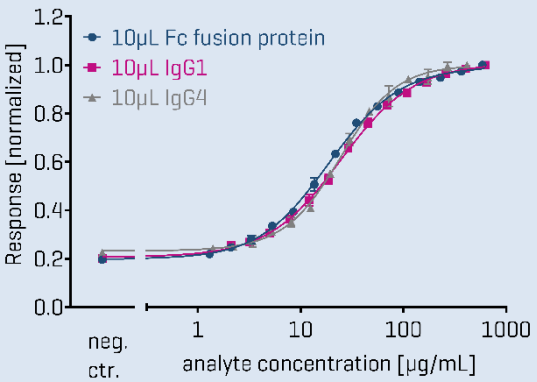

Fig. 3: Calibration curves for IgG1, IgG4 and a Fc Fusion protein

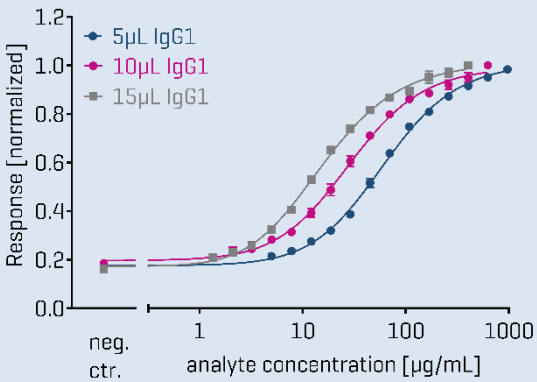

Fig. 4: Calibration curves for different sample volumes

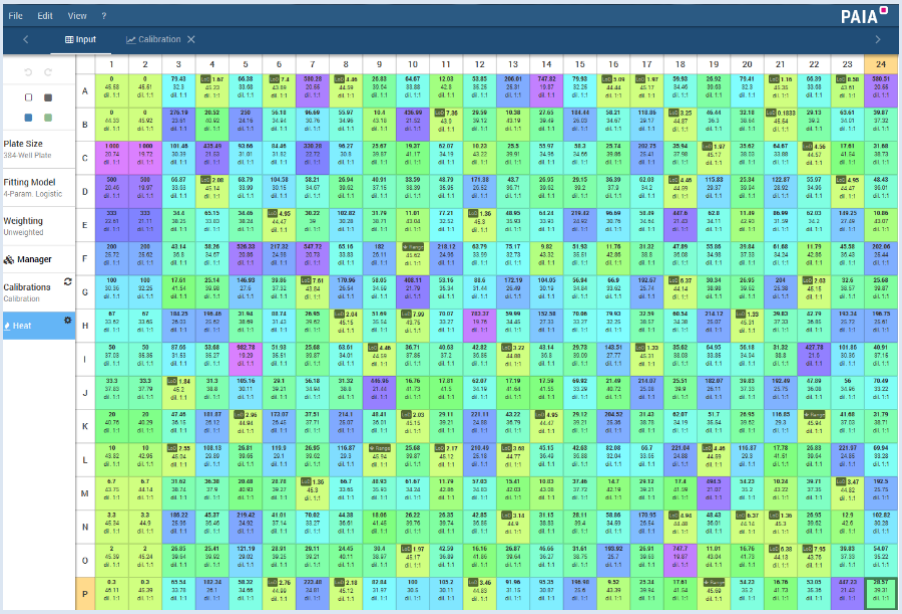

Fig. 5: Result display

A screenshot from the PAIA evaluation software showing a heat map with a calibration curve in duplicate (in clolumns 1 and 2) and results for unknown samples. Wells depicted in purple contain high concentrations of IgG, wells in green correspond to low IgG concentrations.

Conclusion

Running PAIA Fc titer assays on the CELLAVISTA® and NYONE® imagers allows the reliable identification of high producers in very high throughput. The assay range can be easily be adjusted to increase the sensitivity into the range lower than 10 µg/mL analyte.

In addition, the combination of PAIA assays and the CELLAVISTA®/NYONE® imagers makes it possible to monitor cell growth, viability and titer in Fed-Batch cultures in 24- or 96-DWP using only one instrument and one orbital shaker.

Material

- Monoclonal antibodies Rituximab (Mabthera™, Roche) and Mepolizumab (Nucala ™, GSK) and Fc fusion protein Etanercept (Enbrel™, Amgen)
- GIBCO® FreeStyle™ CHO Expression Medium
- Fc titer assay kit PA-104, including PAIA*plate* and Protein A reagent
- Orbital shaker

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