Quantitation of IgG Using PAIA Assays on Cellavista® and NyONE® Imagers

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Application Note
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Abstract

The use of PAIA Human IgG assays on Cellavista® or NyONE® imagers enables the rapid quantification of IgGs in small sample volumes.
This combination provides an efficent way to ensure monoclonality and to monitor cell growth and productivity in early cell line development on just one instrument. Samples for IgG quantification of typically 6 µL can be taken directly from cell culture supernatants and used without dilution.

Introduction

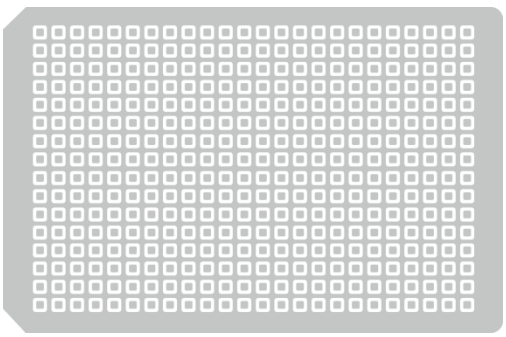
During the cell line development process hundreds to thousands of clones have to be screened to find the most promising, i.e. well growing clones that are also good producers for further development.

The process of single cell cloning is usually performed in 96-well formats and increasingly also in 384-well plates. Thus, there is limited sample volume available for analysis and it is desirable to get the results as quickly as possible.

Since the Cellavista® and NyONE® imagers are established tools to measure the growth and to ensure monoclonality of clones, the combination with PAIA assays also allows the quantification of IgG directly from the supernatant after single cell cloning.

One full 384-well plate is processed in less than one hour and allows for the reliable identification of high producers in an automation-friendly workflow.

Fig. 1: Cellavista® and NyONE® imagers and the schematic representation of a PAIA*plate*



Assay Principle

PAIA assays are fluorescent bead-based immuno-assays that are carried out in single-use 384-well plates. Each of the wells has a transparent protrusion on an otherwise black well bottom. They are used to separate bead-bound analyte-marker complexes from unbound fluorescence marker. Once the reaction between IgG, Protein A beads and fluorescence marker has reached equilibrium, the bead-bound complexes are separated from the solution by sedimentation. Finally, only unbound fluorescence marker is measured through the transparent protrusion on the well bottom (Fig. 2).

The Human IgG Fc/Fab medium titer assay kit (PA 101-01/10) is designed for the rapid quantification of human IgG in sample volumes of 2 – 10 µL for applications like high producer screening in cell line development. It uses Protein A capture beads and a fluorescence marker. While Protein A interacts with the Fc part of IgG, the fluorescence marker of this kit targets the $F(ab')_2$ domain. Thus, the kit is suited for all human IgG classes that interact with Protein A, namely IgG1, IgG2, and IgG4.

Samples with an IgG concentration between 5 and 200 µg/mL can be taken directly from cell culture supernatants and be used in the assay without dilution.

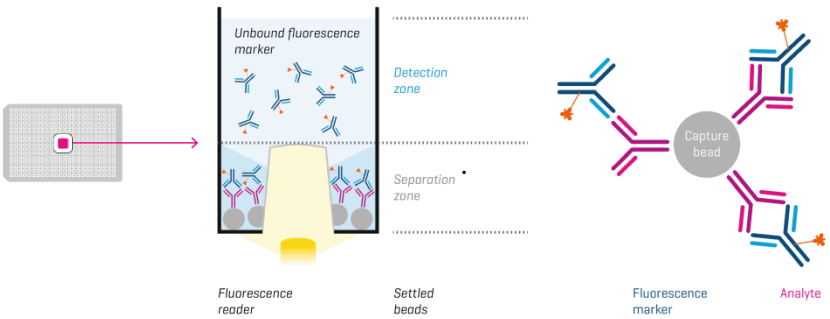


Fig. 2: PAIA assay principle
Protein A coated capture beads, analyte and fluorescence marker are incubated in the wells of a 384-well PAIA*plate*. After sedimentation of the beads with the bound analyte-marker-complexes, the remaining unbound fluorescence marker is measured with the NyONE® or Cellavista®.

Protocol

The Human IgG Fc/Fab medium titer assays were performed according to the standard protocol. First, 54 µL of reaction buffer with fluorescence marker was added to the wells of the PAIA*plate* containing dried Protein A beads. Then, 2 or 6 µL of sample or calibration standard was added.

Calibration standards and samples for recovery experiments for four IgGs were prepared in CHO cell culture supernatant. Calibration standards were measured in triplicates, recovery samples in five replicates. The same experiment was performed on two PAIA*plates*.

The PAIA*plates* were incubated for 45 minutes on an orbital shaker at 1800 rpm. After 15 minutes of bead sedimentation (5 min at 1000 rpm, 10 min without agitation), measurement was performed with the 10x objective at an excitation wavelength of around 640nm (red channel on the NyONE®). Using the NyONE® wizard for PAIA assays, only one image in the centre of each well was taken at a fixed focus position, resulting in a measurement time of 4 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 four monoclonal antibodies show similar shapes with slightly different responses at high IgG concentrations (Fig. 3 and Fig. 4). This reflects the differing affinities of Protein A and fluorescence marker towards the distinct IgG subclasses (Cetuximab, Rituximab and Ofatumumab are IgG1 and Panitumumab is an IgG2 antibody).

Quantitation is most accurate in the concentration range that corresponds to the steep part of the calibration curve (10-60 µg IgG1/mL for 6 µL of sample and 20-125 µg IgG1/mL for 2 µL of sample). The recovery of the samples calculated from the calibration curves is summarized in Table 1 and Table 2.

The CV of the ten replicates (five on each plate) is in the range of 10 %.

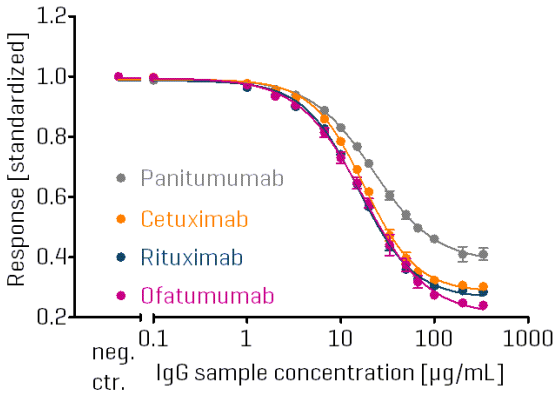


Fig. 3: Calibration curves for monoclonal antibodies from two experiment with three replicates each, 6 µL IgG sample volume

µg/mL	Cetuximab	Rituximab	Ofatumumab	Panitumumab
5	6 ± 1	6 ± 2	6 ± 0	7 ± 1
10	11 ± 3	9 ± 1	10 ± 1	12 ± 3
20	17 ± 4	18 ± 2	19 ± 2	24 ± 1
40	35 ± 3	36 ± 3	36 ± 4	44 ± 2
80	67 ± 8	59 ± 4	70 ± 7	69 ± 5

Table 1: Recovery of unknown samples, 6 µL volume
Data is shown as mean ± standard deviation and was obtained in two independent experiments with five replicates each.

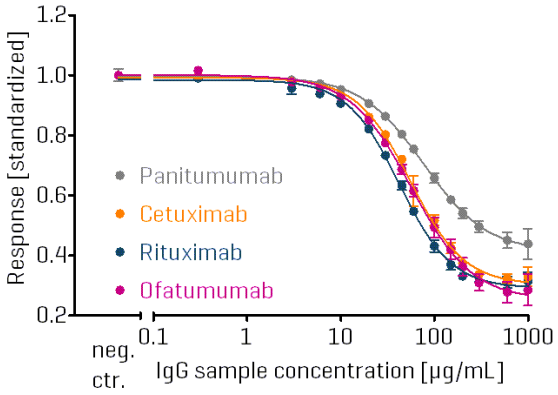


Fig. 4: Calibration curves for monoclonal antibodies from two experiments with three replicates each, 2 µL IgG sample volume

µg/mL	Cetuximab	Rituximab	Ofatumumab	Panitumumab
16	16 ± 1	14 ± 2	21 ± 1	23 ± 2
31	29 ± 1	27 ± 1	36 ± 2	44 ± 3
63	56 ± 2	53 ± 2	65 ± 2	85 ± 4
125	119 ± 8	111 ± 11	126 ± 10	172 ± 7
250	241 ± 21	223 ± 56	286 ± 53	284 ± 42

Table 2: Recovery of unknown samples, 2 µL volume
Data is shown as mean ± standard deviation and was obtained in two independent experiments with five replicates each.

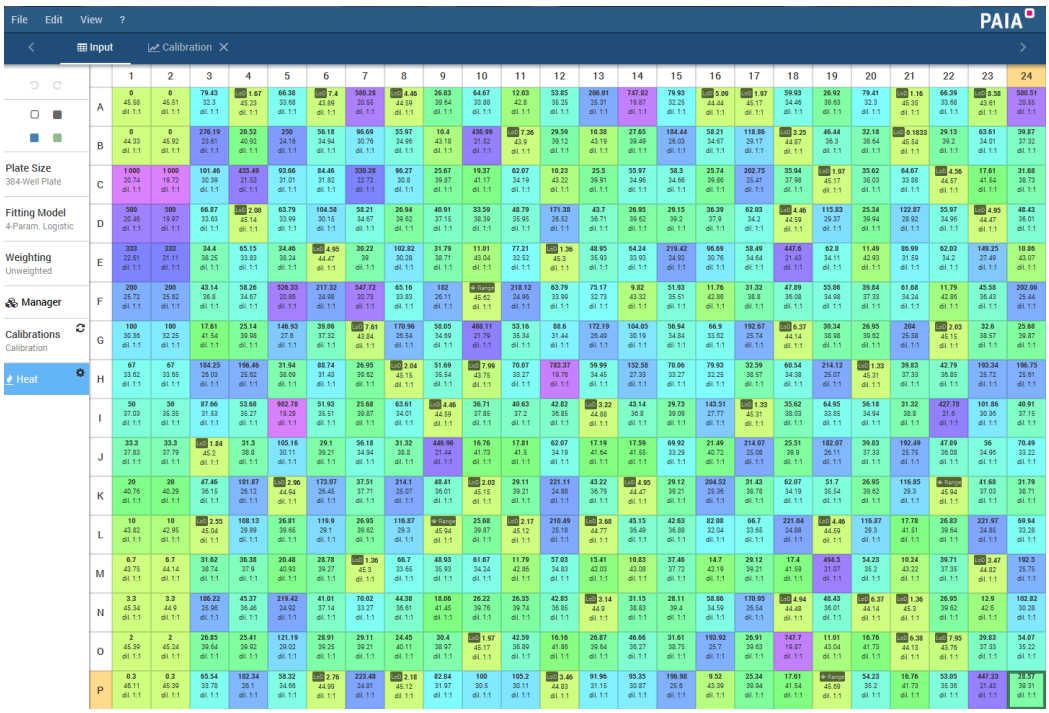


Fig. 5: Result display
A screenshot from the PAIA evaluation software showing a heat map with a calibration curve in duplicate on the left hand side and results for unknown samples. Wells depicted in purple contain high concentrations of IgG, wells in green correspond to low IgG concentrations.

Conclusion

Running PAIA IgG quantification assays on the Cellavista® and NyONE® imagers allow the reliable identification of high producers with very limited amount of samples from cell culture supernatants and high throughput.

With the combination of PAIA assays and the Cellavista®/NyONE® imagers it is possible to monitor monoclonality, cell growth and productivity of clones after single cell seeding using only one instrument. The workflow is amenable to automation and offers a high throughput which can be increased to up to four 384-well plates per hour if several shakers are used in parallel. It is also possible to use shakers that can shake several plates.

Material

Material:

- Monoclonal antibodies Rituximab, (Mabthera™, Roche), Cetuximab (Erbitux™, Merck), Ofatumumab (Arzerra™, GSK) and Panitumumab (Vectibix™, Amgen)
- PAIA Human IgG Fc/Fab kit PA 101-01/10, including PAIA*plate* and reaction buffer
- Orbital shaker (min 1800 rpm)
- Supernatant from CHO cells cultivated in DMEM/Ham's F12 (PAN-Biotech)