APPLICATION NOTE

Streamline the workflow for measuring IgG in cell line development

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Benefits

- **Easy data acquisition and analysis with preconfigured SoftMax Pro Software protocol**
- **Fast, homogeneous assay with results in less than an hour**
- **Precise measurement of IgG from 2.5 to 100 mg/L**

Introduction

The process of cell line development for the production of antibody-based therapeutics can vary widely amongst researchers. The ultimate goal is to find a clone that stably produces large quantities of a high-quality monoclonal antibody.

The measurement of IgG production is a crucial step at many stages in the development and manufacturing of monoclonal antibodies (Figure 1). Commonly used methods for IgG quantitation require either special instrumentation and skilled personnel, such as HPLC and surface interferometry, or time-consuming assays like ELISA (enzyme-linked immunosorbent assay). Although the ELISA is a well-established method for protein quantitation, it is a lengthy, multi-step process (Table 1). Here, we introduce the use of the Valita®TITER assay from Valitacell for measuring IgG titers during the antibody development and manufacturing process.

The ValitaTITER Assay measures IgG concentrations from 2.5 – 100 mg/L with a simple add-and-read protocol. The assays are performed in less than one hour and can be incorporated into the bioprocess workflow in a 96 well plate format. The assay is high throughput and can be fully automated. Analysis can be carried out in cell culture media with a low sample volume and without complex preparation steps. Assay detection can be performed on multi-mode microplate readers from Molecular Devices: SpectraMax® iD5 Multi-Mode Microplate Reader, SpectraMax® M5 Multi-Mode Microplate Reader, and SpectraMax® i3x Multi-Mode Microplate Reader with the Fluorescence Polarization Detection Cartridge.

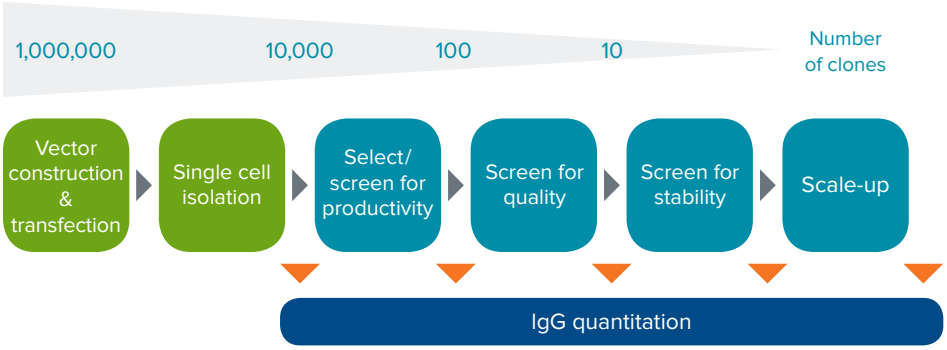

Figure 1. The cell line development process from cell transfection to scale-up.

	ValitaTITER	Surface Interferometry	ELISA	HPLC
Total assay time (96 samples)	45 min	55–65 min	6+ hours	25–45 hours
Measurement range (mg/L)	2.5–100	0.025–2000	0.5–5	> 10
Sample volume	5 µL–30 µL	180 µL +	100 µL	1–2 mL
Precision	< 2%	< 5%	< 5–10%	< 2%

Table 1. Performance of ValitaTITER assay compared to other methods used for IgG quantitation.

Assay principle

The ValitaTITER assay is based on detection of IgG Fc interactions with Protein G using fluorescence polarization (FP) technology. Each well of a 96-well microtiter plate is coated with a fluorescently labeled IgG-binding peptide, Protein G. When samples are added to the wells, the Protein G molecules are resuspended and binding occurs. The rate of molecular motion of Protein G slows down when it is bound to antibodies, resulting in an increase of the FP value (Figure 2).

Materials

- ValitaTITER Assay Kit (Valitacell cat. #00010)
- IgG protein standard (Sigma cat. #I2511)
- CD CHO Medium (Thermo Fisher Scientific cat. #10743011)
- In-process samples: cell culture supernatant samples generated by recombinant IgG producing CHO cell lines cultivated in suspension
- SpectraMax iD5 Multi-Mode Microplate Reader equipped with 535-nm FP emission filters
- SpectraMax i3x Multi-Mode Microplate Reader with Fluorescence Polarization (FP-FLUO) Detection Cartridge
- SpectraMax M5 Multi-Mode Microplate Reader

Methods

IgG standards were serially diluted in CD CHO cell culture medium to concentrations between 2.5 and 100 mg/L. A range of IgG samples of cell culture supernatant were diluted as required in cell culture media. 60 µL of ValitaMab reconstitution buffer was pipetted into each well of the ValitaTITER plate, followed immediately with 60 µL of prepared cell samples or protein standards. Contents were mixed and incubated in the dark for 30 minutes at room temperature. FP signal was measured on the SpectraMax iD5 reader using the onboard monochromator optics for excitation and a 535-nm filter set for emission. Data were generated and analyzed with a preconfigured SoftMax Pro protocol (Figure 3). FP was also measured on SpectraMax i3x and SpectraMax M5 readers to verify their performance (data not shown).

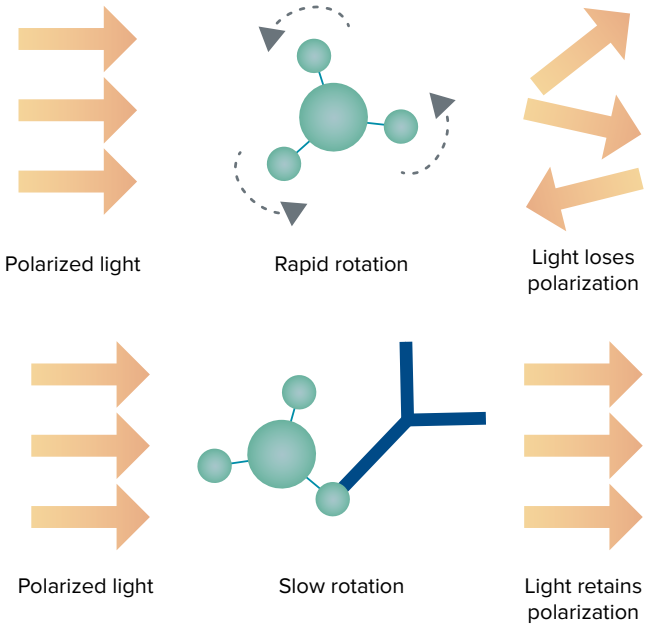

Figure 2. ValitaTITER assay principle using the FP technology. Free Protein G molecules are smaller and rotate faster in the buffer solution. As a result, the polarized excitation light loses polarization **(top)**. When Protein G molecules bind to the antibodies in the samples, the rotation of the larger complex slows down resulting in increase of the FP values **(bottom)**.

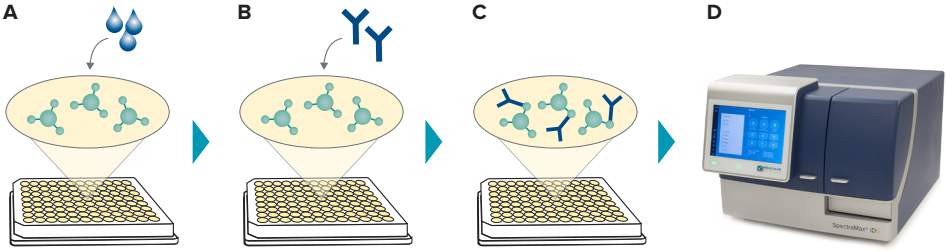

Figure 3. ValitaTITER assay workflow. (A) ValitaMab reconstitution buffer is pipetted into each well of the ValitaTITER plate. **(B)** Samples or IgG standards are added to the wells. **(C)** The plate is incubated for 30 min to allow binding to occur. **(D)** FP is measured on the SpectraMax iD5 reader using SoftMax Pro Software.

Comparison data were generated using Protein A HPLC. Experiments were carried out according to the manufacturer's instructions.

Results

With the ValitaTITER assay, an IgG standard curve was obtained using a simple add-and-read method with no wash steps and a small (5-30 µL) sample volume. On the SpectraMax iD5 reader, best results were obtained using built-in monochromator optics for excitation and a 535-nm FP filter set for emission. Standards from 3.1 to 100 mg/L were detected with a high degree of linearity (r^2 = 0.998) across the entire range (Figure 4). Similar data were obtained with the SpectraMax i3x and SpectraMax M5 readers (data not shown). A preconfigured protocol in SoftMax Pro Software automated the mP calculations and curve plotting.

Results from the ValitaTITER assay for conditioned media samples from fed-batch reactors were compared to HPLC. The two methods showed a strong correlation, with r^2 = 0.994 (Figure 5).

Conclusion

IgG quantitation must be performed at many steps in the cell line development process to ensure a high-quality final product for scale-up, so an assay that enables accurate results with a minimal investment of time is critical to success. Here we demonstrate that the IgG quantitation data obtained from the ValitaTITER assay matched those from HPLC analysis, widely regarded as a gold-standard method, but were generated in less than 3% of the time required for HPLC.

The ValitaTITER assay is a homogeneous, high-throughput method for precise and rapid quantitation of IgG in the cell line development workflow. This 96-well assay has been fully validated on the SpectraMax iD5 reader and other Molecular Devices plate readers with FP detection to ensure reliable results. SoftMax Pro Software minimizes setup time for detection and automates standard curve fitting and sample quantitation.

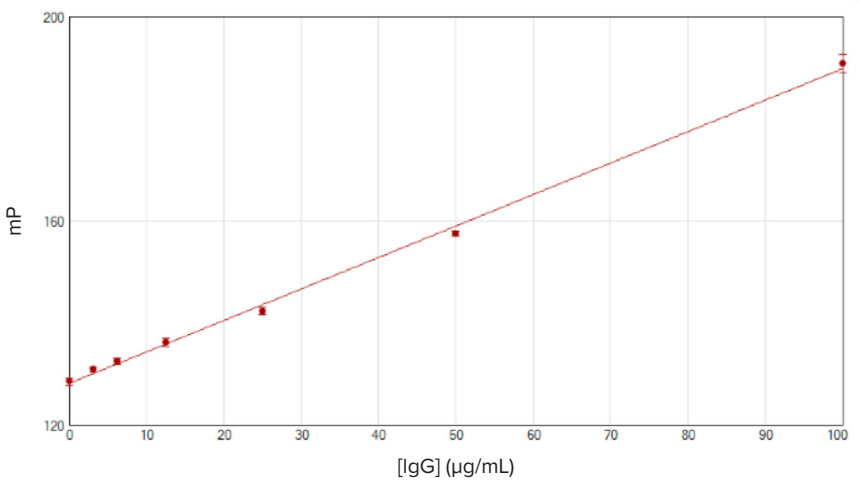

Figure 4. IgG standard curve generated using the ValitaTITER assay on the SpectraMax iD5 plate reader. Data were plotted using a linear curve fit in SoftMax Pro software (r^2 = 0.998). Error bars show standard deviation of 6 replicates.

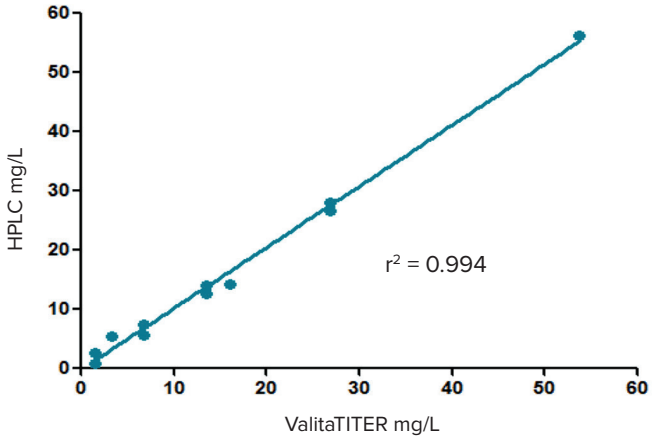

Figure 5. Comparative analysis of IgG quantitation by ValitaTITER assay and Protein A HPLC analysis. IgG titer in a range of conditioned media samples from fed-batch bioreactors was quantified by ValitaTITER fluorescence polarization assay. The samples were also analyzed by Protein A HPLC.

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