Fluorescence Polarization based assay for rapid, precise, high-throughput measurement of IgG & Fc containing derivatives

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- **BMG LABTECH microplate readers enable measurement of novel IgG quantification method**
- **Valita™TITER assay uses fluorescence polarization to determine IgG directly in cell culture supernatant**
- **Preconfigured measurement protocols and analyses facilitate and accelerate quantitation**

Introduction

The accurate, rapid and high-throughput measurement of IgG is essential in the development and manufacture of most therapeutic antibodies. Monoclonal antibodies are becoming increasingly dominant in biopharmaceuticals, where a vast number of samples must be screened for the development of each potential therapeutic. Here we present the Valita™TITER assay, a novel assay for the quantification of IgG in cell culture supernatant.

Current workflows, which include HPLC protein A, ELISA, protein A surface interferometry, and HTRF (homogenous time resolved fluorescence) have major drawbacks, which include costly, labour-intensive methods and long analysis times.

The Valita™TITER assay provides a very accurate and cost-effective solution to measure IgG in a range from 2.5-80mg/L. The Valita™TITER assay relies on the detection of IgG Fc interactions with Protein G by measurement of fluorescence polarization (Fig. 1). The assay comes in a 96-well format and is simple, high-throughput, rapid and fully automatable. Analysis can be carried out in cell culture media with a low sample volume and no complex preparation steps.

In this application note, we show the optimal protocol for performing IgG quantification using Valita™TITER assay kits obtained by the PHERAstar®, POLARstar® Omega and the CLARIOstar® plate readers.

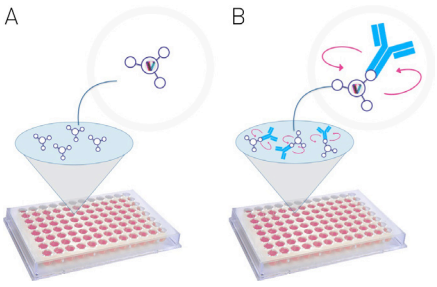

Fig. 1: Assay schematic of Valita™TITER assay for IgG quantification using fluorescence polarization: Each well of the 96-well Valita™TITER plate is pre-coated with a fluorescently labelled IgG binding peptide, protein G (A). IgG sample binds to the peptide and binding is measured via fluorescence polarization and rotational diffusion (B).

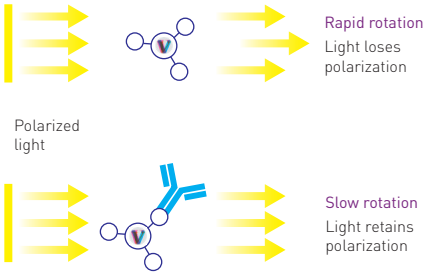

Fig. 2: Assay Principle: The assay applies fluorescence polarisation to quantify IgG. Concentration is measured by detection of changes in light polarisation caused by molecule rotation. Small, unbound molecules rotate rapidly in solution, while large, bound molecules rotate slowly.

FP effectively analyzes changes in the size of molecules. "Fixed" fluorophores that are excited by polarized light preferentially emit light in the same plane of polarization. However, rotation of the molecules between absorption and emission of the photon has the effect of "twisting" the polarization of the light. Small molecules tumble faster in solution than larger molecules. Hence, the change of size of molecules, with an associated fluorophore, can be measured using the degree of light depolarization. Consequently, when fluorescently labelled Protein G is unbound, it tumbles rapidly and depolarizes the light more than when it is bound to an IgG (which is 5 times larger) (Fig. 2). This change in polarization is used to measure the amount of IgG in the solution. FP is measured by exciting the solution with plane polarized light and measuring the intensity of light emitted in the plane parallel to the exciting light (polarized proportion) and perpendicular to the exciting light (depolarized portion). The FP is expressed as a normalized difference of these two intensities, which is typically in millipolarization units (mP).

Materials & Methods

- Valita™TITER Assay kit
- Valita™APP software (provided)
- IgG standard (IgG from Human Serum)
- PHERAstar, POLARstar Omega, and CLARIOstar (BMG LABTECH)

Experimental Procedure

Samples were prepared according to the respective product instruction for use. A standard curve was prepared using

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an IgG standard as per IFU. 60 µl of ValitaMab reconstitution buffer was pipetted into each well of the Valita™TITER plate, along with 60 µl of prepared standards and samples. Contents were mixed and incubated in the dark for 30 minutes before being read on the POLARstar, PHERAstar, and CLARIOstar plate readers using preconfigured protocols in the BMG LABTECH software (detailed settings below). Data was then analysed using exported .csv files from the raw data, using the ValitaAPP software.

Instrument settings

	PHERAstar	CLARIOstar	POLARstar Omega
Optic settings	Fluorescence Polarization, endpoint		
	Filters		
	Optic module FP 485 520 520	Ex: 482-16 Dichroic: LP504 Em: 530-40	Ex: 485 Em: 520
	Focus and gain adjusted prior to measurement (sample: 0 mg/ml)		
	70 mP target mP		
General settings	200 flashes per well		
	0.5 s settling time		

Results & Discussion

A standard curve (2.5-80 mg/l) was quantified with the Valita™TITER assay on three FP capable multi-mode readers by BMG LABTECH (POLARstar Omega, CLARIOstar and PHERAstar). All readers showed low variation of replicate measurements (Fig. 3).

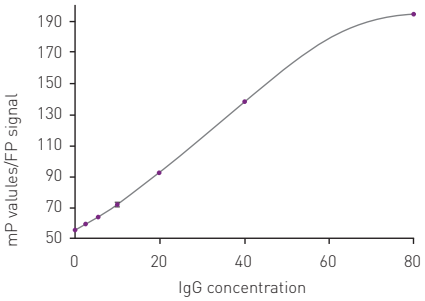

Fig. 3: Standard curve of IgG quantified with the Valita™TITER assay on the CLARIOstar microplate reader. Error bars show standard deviation of triplicates.

Finally, the Valita™TITER IgG quantification assay was compared to a conventional IgG quantitation by HPLC and used a range of differentially conditioned cell culture media samples. Figure 4 shows the correlation of both methods and reveals similar precisions for both quantification assays with Valita™TITER being much faster due its homogenous format with no need for complex sample preparation.

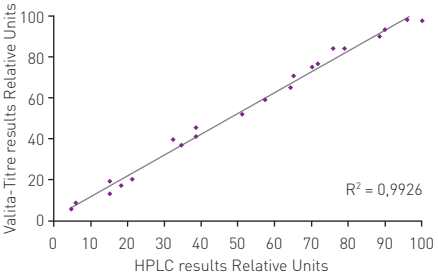

Fig. 4: Comparison of IgG quantification with Valita™TITER assay and by HPLC.

Conclusion

The ability to quickly determine IgG concentration from a mixed sample is of particular importance and value during the development and manufacture of these biopharmaceuticals. Here we report a novel, rapid, and simple fluorescence polarization based assay for high-throughput titer measurement of IgG and FC-containing derivatives. The Valita™TITER assay allows the direct quantification of IgG from process samples without sample preparation or purification steps. The CLARIOstar, PHERAstar and POLARstar exhibit excellent assay quality when used with the Valita™TITER assay. By comparison with alternatives, Valita™TITER is a high-throughput, simple, precise method for quantification of IgG.

References

1. Thompson, B. et al. (2017) High-throughput quantitation of Fc-containing recombinant proteins in cell culture supernatant by fluorescence polarization spectroscopy, *Analytical Biochemistry* **534**: 49-55.

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