Rapid & High-Throughput IgG Quantification in Cell Culture Supernatant Using ValitaTiter Microplates

Eligio Iannetti, Brian Murphy, Hannah Byrne; support@valitacell.com; ValitaCell Ltd, Dublin, Ireland.

ValitaTiter Assay Overview

The accurate, rapid and high-throughput measurement of immunoglobulins, particularly immunoglobulin G (IgG), is essential in the development and subsequent manufacture of therapeutic antibodies. Here we describe the ValitaTiter assay, a rapid, robust and accurate IgG quantification assay. The ValitaTiter assay range measures IgG concentrations from 2.5 to 2000 mg/L, with a simple add-mix-read protocol. The assay is performed in less than 15 minutes and can be incorporated into any bioprocess workflow in a 96- or 384-well plate format. The assays are high throughput and can be fully automated. Analysis can be carried out in crude cell culture media containing up to 15 x 10^6 cells/mL with a low sample volume and limited test sample pre-preparation. Assay detection can be performed using fluorescence polarization on a standard microplate reader.

ValitaTiter Assay Principle

The ValitaTiter assay quantifies IgG-Fc interactions with a fluorescently labelled derivative of protein G using fluorescence polarization (FP) for detection. FP effectively analyses changes in the size of molecules (Figure 1).

When you excite a sample with plane polarized light, the probe unbound in solution rotates rapidly leading to depolarization of emitted light. When bound to a higher molecular weight target protein, the complex rotates slowly, leading to the retention of polarized light. The amount of target in solution can then be calculated, with the observed output polarization in a mixture of labeled probe and target being proportional to the fraction of bound probe in solution.

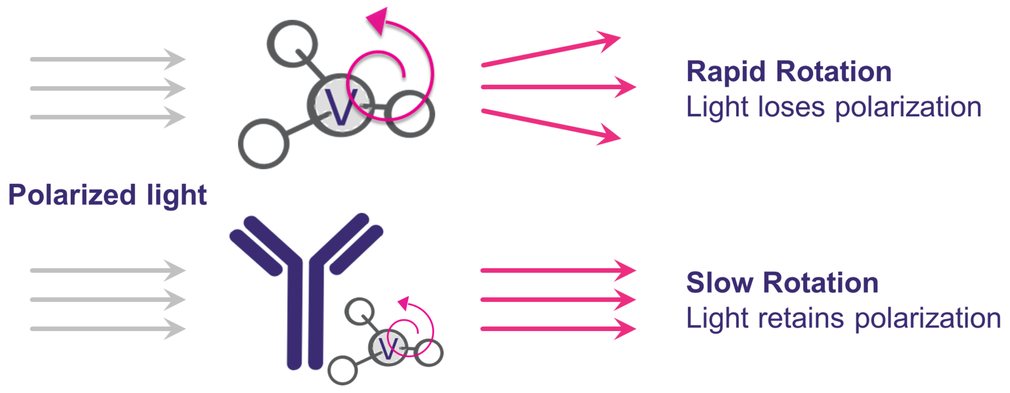
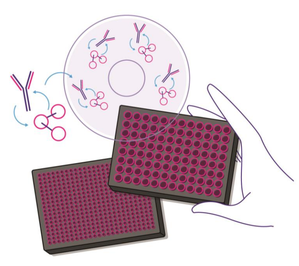


Figure 1: Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

ValitaTiter Assay Workflow: Add, mix and read

The ValitaTiter assay workflow consists of 3 simple steps. The ValitaTiter plates come pre-coated with an IgG-Fc-specific fluorescently labelled probe. The probe is reconstituted using fresh cell culture media. Following this, IgG standard or test sample is added to the plate. After a short incubation period, the plate is measured using a plate reader with FP. Depending on the plate reader and method, results can be obtained for 96 or 384 crude samples in less than 15 minutes.



Add fresh media & IgG sample and mix

Incubate for 5 minutes at room temperature

Measure IgG using FP on a plate reader

Figure 2: The simplicity, speed & throughput of the ValitaTiter assay make it ideal for both manual & automated workflows.

ValitaTiter Assay Benefits

In addition to the simplicity & speed of the ValitaTiter assay, it is robust to cell contamination facilitating crude IgG sample analysis straight in crude samples from cell culture. This eliminates the need for any sample preparation such as filtration or centrifugation to remove cells or purification. Users can simply sample directly from culture to ValitaTiter assay plates & read.

The ValitaTiter assay also compares well to industry gold standard techniques for IgG titer measurement.

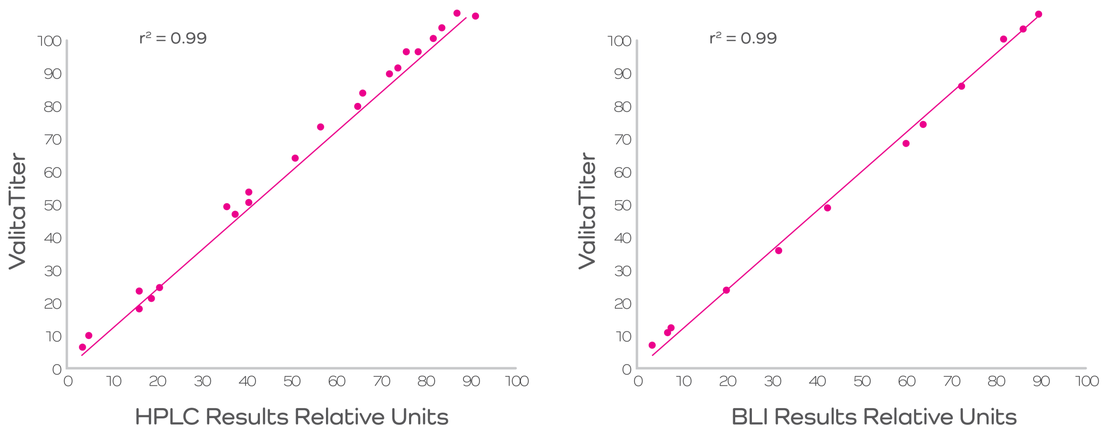


Figure 3: IgG standard curves quantification. Correlation analysis by the ValitaTiter assay, Protein A HPLC and BLI methods.

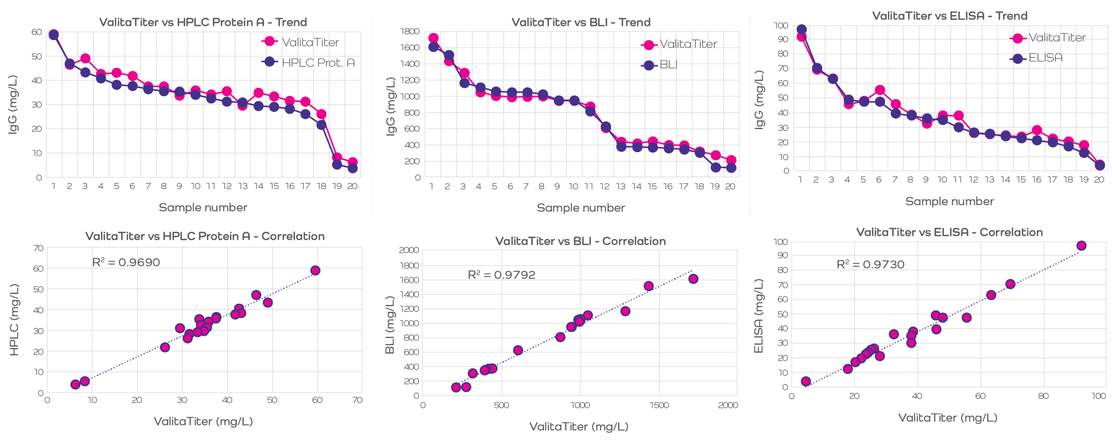


Figure 4: IgG quantification in crude cell culture samples. Trend and correlation analysis by the ValitaTiter assay, Protein A HPLC, BLI and ELISA methods.

In summary, the ValitaTiter assay range has considerable advantages over alternative quantification assays including cost, simplicity, speed and throughput.

	ValitaTiter	BLI	ELISA	HPLC
Assay time	**15 mins**	1 hour	5 hours	10 hours
Sample prep	**none**	none	cell centrifugation	cell centrifugation and protein purification
Reagents	**1**	>2	>6	2
Steps	**add, mix, read**	>3	>20 steps	>5
Costs	**$**	$$$$	$$	$$$

Figure 5: Overview of the key features of the ValitaTiter assay in comparison to industry standard IgG quantification techniques.

Conclusion

The ValitaTiter assay is a simple, accurate, rapid & automation-friendly assay that enables high-throughput quantification of IgGs from crude samples.

The ValitaTiter assay is robust to cell contamination and facilitates 'straight from cell culture measurement' across a broad functional range.

The ValitaTiter assay has considerable advantages over alternative quantification assays including cost, simplicity, speed and throughput.

2022-GBL-EN-100353-v1