Rapid & High-Throughput IgG Quantification and Protein Aggregation Screening in 96-well or 384-well plates

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Overview

The accurate, rapid and high-throughput measurement of immunoglobulin G (IgG) and protein aggregation is essential in the development and subsequent manufacturing of therapeutic antibodies.

Valita®Titer & Valita®Aggregation are 96 and 384-well assays that enable simple, accurate and high-throughput quantification of IgG or protein aggregation in solution. Both the assays consist of a simple add-mix-read procedure that, depending on the workflow, can measure 96 samples in less than 15 minutes using a standard microplate reader. Valita®Titer has been benchmarked against industry gold standard techniques such as HPLC. Studies are ongoing to demonstrate the use of Valita®Aggregation alongside technologies like HPLC-SEC.

Assays Principle

Valita®Titer & Valita®Aggregation assays quantify IgG & protein aggregate interactions with fluorescently labelled probes using fluorescence polarisation (FP) for detection. FP effectively analyses changes in the size of molecules (Figure 1).

When you excite a sample with plane polarised 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 polarised light. The amount of target in solution can then be calculated, with the observed output polarisation in a mixture of labeled probe and target being proportional to the fraction of bound probe in solution.

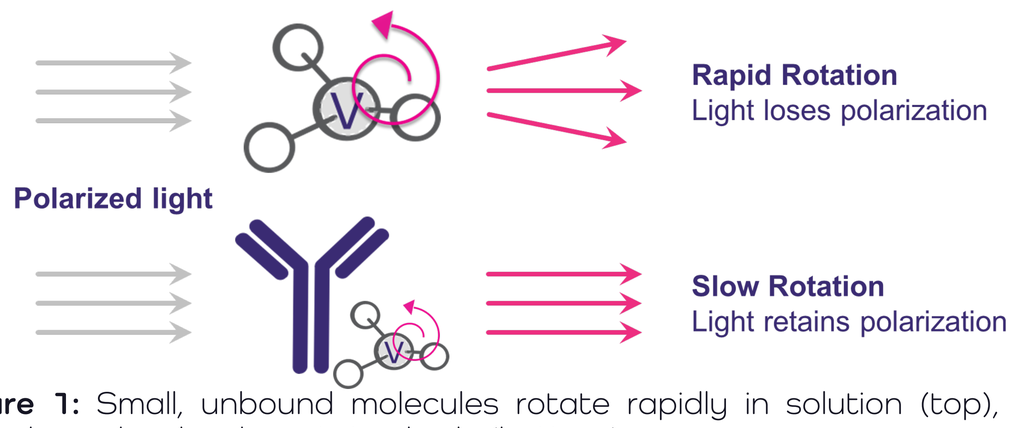

Figure 1: Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

Workflow: Add, mix and read

Valita®Titer & Valita®Aggregation workflows consist of a simple add-mix-read procedure. The plates come pre-coated with fluorescently labelled probes. The probes are reconstituted in the different plates using fresh cell culture media (Valita®Titer) or PBS (Valita®Aggregation). Following this, 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 workflow, results can be obtained from crude samples in less than 15-minutes total assay time.

Figure 2: The simplicity, speed & throughput of the Valita®Titer & Valita®Aggregation plates make these ideal for both manual & automated workflows.

Outputs & Benefits

In addition to the simplicity & speed of Valita®Titer, the assay is robust to cells, pH and media variation allowing sampling direct from the bioreactor. Comparative data with competing technologies demonstrate that the assay correlates well with gold standard techniques such as HPLC protein A and BLI (figure 3).

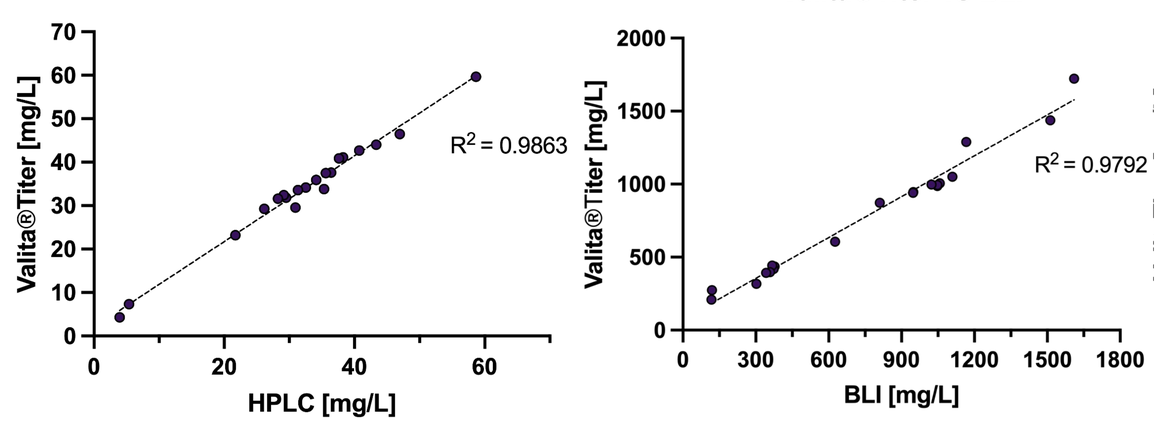

Figure 3. IgG titer quantification in crude cell culture samples.
Trend and correlation analysis of Valita®Titer versus competing technologies.

Valita®Aggregation represents a high-throughput aggregation screening tool demonstrated to accurately detect non-monomer in purified samples (figure 4). Validation studies are ongoing for the deployment of this product for screening crude samples.

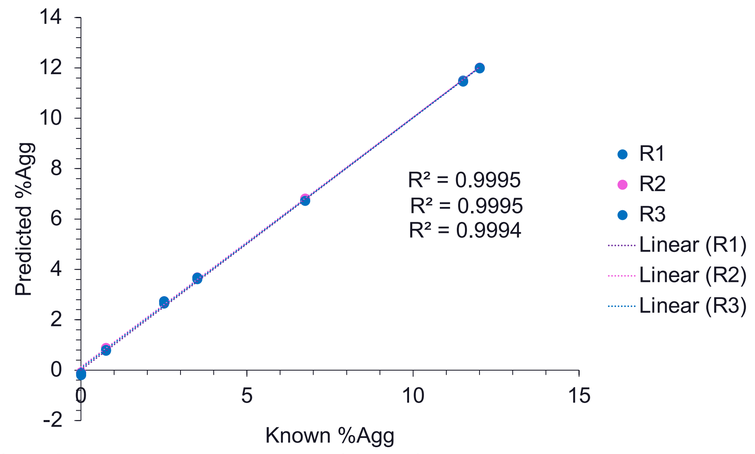

Figure 4. Valita®Aggregation predicted % aggregation [y-axis] versus known % aggregation [x-axis]. A high degree of correlation and reproducibility observed between the predicted values and known values.

In summary, Valita®Titer & Valita®Aggregation assays have considerable advantages over alternative antibody screening tools including cost, simplicity, speed and throughput.

	Valita®Titer & Valita®Agg	HPLC
assay time	15 mins	10 hours
sample prep	none	cell centrifugation and protein purification
reagents	1	3
steps	add, mix, read	>5
costs	$	$$$

Figure 5: Overview of the key features of the Valita®Titer assay in comparison to the industry standard HPLC method.

Valita®Titer & Valita®Aggregation Conclusion

- ValitaCell plates are simple, accurate, rapid & automation friendly, enabling high-throughput antibody screening for IgG titer and aggregation
- Valita®Titer plates are robust to cell contamination and facilitate 'straight from cell culture' quantification.
- ValitaCell plates have considerable advantages over alternative quantification assays including cost, simplicity, speed and throughput.