BECKMAN COULTER
Life Sciences

Increased throughput for IgG quantification using the Valita Titer 384-well plate

Introduction

The Valita Titer assay is a rapid, high-throughput IgG quantification assay which uses fluorescence polarization (FP) for detection and relies on interactions between a fluorescently labeled IgG Fc-specific probe and the Fc of an IgG. FP effectively analyzes changes in the size of molecules, given that smaller molecules tumble more rapidly than larger ones in solution. The rotation of the molecules between absorption and emission of the photon has the effect of "twisting" the polarization of the light. When the fluorescently labeled IgG-binding peptide is unbound, it tumbles rapidly and depolarizes the light more than when it is bound to an IgG (which is about 20 times larger). Therefore, 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). FP is expressed as a normalized difference of these two intensities, which is typically in millipolarization units (mP).

Product Features

The Valita Titer 384-well assay represents an expansion of a pioneering quantum technology platform from Beckman Coulter Life Sciences and the Valita Titer assay range. It enables researchers to further increase their throughput for IgG quantification, with the ability to analyze up to 4X the number of samples per plate, using 33% less sample volume, versus the traditional Valita Titer 96-well assay. The 384-well format and the 'add-mix-read' nature of the assay, with no requirement for sample pre-preparation or wash steps, makes it ideal for incorporation into a partially or fully automated bioprocess workflow. Assay detection can be performed on a range of multi-mode microplate readers with fluorescence polarization detection capabilities. Unlike other IgG quantification methods, the Valita Titer 384-well plate can deliver precise, homogeneous measurement of IgG from 2.5 – 100 mg/L in less than 15 minutes. An additional Valita Titer Plus 384-well product (VAL014) is also available with an extended concentration range (100 – 2000 mg/L).

Assay Principle

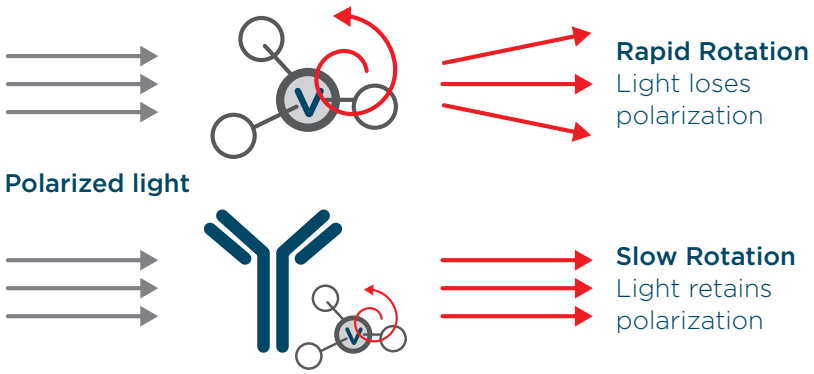

FIGURE 1. Valita Titer assay principle. Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

Materials and Method

Materials

- Valita Titer 384-well plate, catalogue number: VAL013, detection range: 2.5 - 100 mg/L
- CD CHO medium (Gibco™, Catalogue No. 10743)
- BMG Labtech PHERAstar Multimode plate reader
- Human IgG1 kappa
- ThermoFisher Finnpipette F2 Pipettes (Catalogue No. 10413865, 1187735, 11887351, 4662060)
- Starlab TipOne Tips (0.5 - 200 µL Catalogue No. S1111-1700) (1000 µL Catalogue No. S1111-6811)

Method

The instrument settings used for assay plate measurement are summarized in Table 1 and are identical to the 96-well product, with the exception of the plate type.

Plate type	Corning/Costar 384-well Black Flat Bottom, #3573
Optic settings	Fluorescence Polarization, endpoint
	Optic module FP 485 520 520
	Focus and gain optimized from most fluorescent well (0 mg/L)
	70 mP target mP for gain
General settings	200 flashes per well
	0.5 s settling time

TABLE 1. BMG Labtech Pherastar optimized instrument settings.

Assay Procedure

1. 40 µL of cell culture media was added to each well to reconstitute the Fc-specific probe (pre-dried onto the surface of the Valita Titer 384-well assay plate).
2. 40 µL of each standard/sample was then added into appropriate wells of the 384-well plate.
3. A multichannel pipette was used to mix each well 3 times prior to a 5-minute incubation in the dark.
4. Post-incubation, the plate was measured using FP.

FIGURE 2. Workflow schematic of Valita Titer assay.
1. Add fresh media and IgG sample and mix.
2. Incubate for 5 minutes at room temperature.
3. Measure IgG using fluorescence polarization on a plate reader.

Results

The performance of Valita Titer 384-well assay to accurately determine the concentration of IgG in cell culture media was assessed. As this is a relative quantification method, to enhance the accuracy of predication, it is important to ensure that the molecule deployed as the standard is homogeneous to the test samples. In this example, when quantifying human IgG1 kappa test samples, a human IgG1 kappa standard curve was used for interpolation. An eight-point IgG1 kappa standard curve (0 – 100 mg/L) was prepared in triplicate and analyzed using the Valita Titer 384-well plate. Alongside standard curve generation, samples of known concentration were interpolated against the standard curve and accuracy bias assessed.

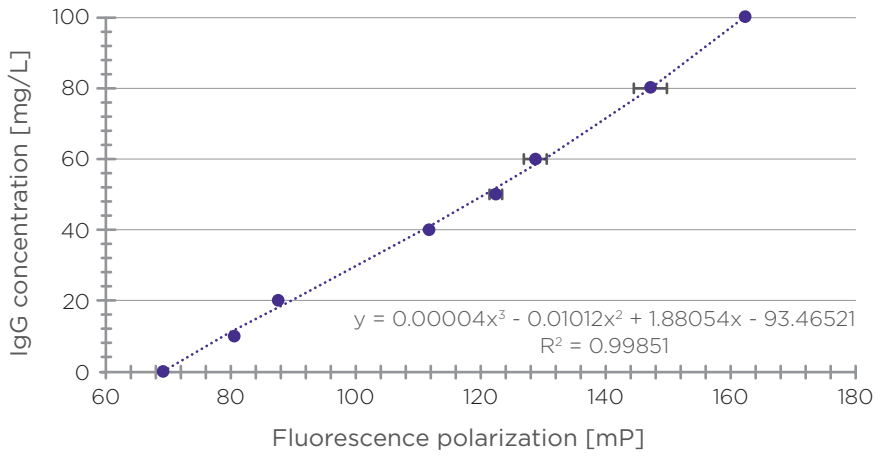

FIGURE 3. Valita Titer assay standard curve plotted using a cubic fit (poly-3 fit) ($R^2 = 1$).

A 3rd order polynomial fit was identified as the optimum fit for the standard curve samples to allow for the most accurate interpolation. Here, the concentration of IgG [mg/L] is plotted on the y-axis and the Raw FP on the x-axis [Figure 3]. The equation of the line was utilized to interpolate the concentration of IgG test samples by substituting the output Raw FP value for x and solving for y. The assay accurately interpolated the known concentration of test samples with an accuracy bias of ≤10% versus the known absolute concentration. The assay also demonstrates excellent reproducibility with a standard deviation between replicate samples of ≤2 mP and an intra-plate coefficient of variation [CV] of ≤2% [Table 2]. Figure 4 demonstrates that the predicted concentration of IgG test samples generated using the Valita Titer 384-well assay correlates well with the absolute values, with an $R^2 < 0.99$.

Known IgG conc in sample (mg/L)	Interpolated conc rep1 (mg/L)	Interpolated conc rep2 (mg/L)	Interpolated conc rep3 (mg/L)	Average (mg/L)	Std. deviation (mP)	Accuracy bias (%)	Coefficient of variation (%)
90	90.4	89.8	89.2	89.4	0.71	-1	0.456
80	79.9	78.5	80.6	79.7	0.85	1	0.578
70	75.2	70.8	71.6	72.8	1.63	4	1.152
60	56.3	57.2	57.4	56.8	0.95	-5	0.749
50	51.1	53.5	50.5	51.7	1.51	3	1.232
45	42.1	41.9	42.1	41.9	0.69	-7	0.615
40	40.8	42.1	39.9	40.9	1.14	2	1.023
25	24.3	24.9	24.1	24.0	0.78	-4	0.835
20	19.1	18.4	18.3	18.1	0.74	-9	0.838

TABLE 2. Summary of output data from the use of Valita Titer 384-well product for quantifying IgG test samples of known concentration.

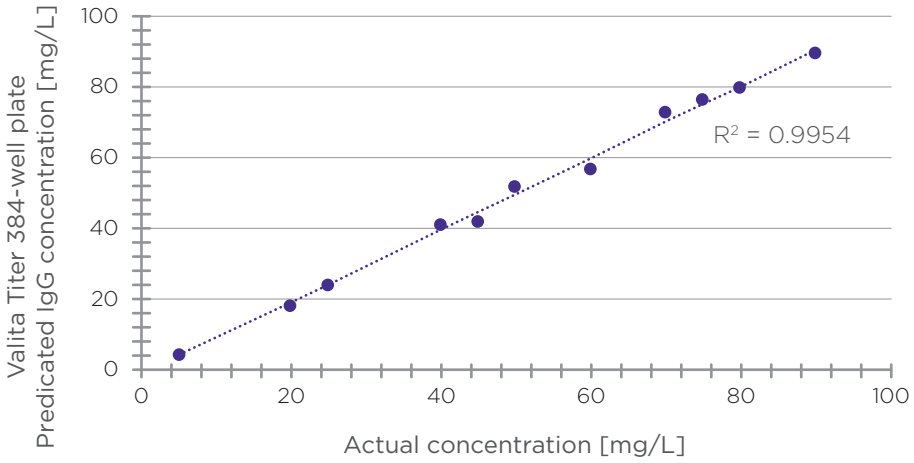

Figure 4. The Valita Titer assay predicted IgG concentrations plotted against the concentrations for canine polyclonal IgG (R^2 = 0.9997).

Conclusions

The data presented here demonstrates that the Valita Titer 384-well assay retains all the key features of its 96-well counterpart and is capable of robust, rapid and accurate measurement of IgG samples and standards in solution. The assay allows for high-throughput, direct and precise quantification of crude and purified IgG samples at various stages of drug development. With the expansion of automated and liquid handling robot-based systems in the bioprocessing industry, the Valita Titer 384-well product represents a key tool which can be easily integrated into these platforms to allow for maximum productivity where rate of throughput is a key priority for users.

Abbreviations

FP Fluorescence polarization

mP Millipolarization units

IgG Immunoglobulin G

About the Authors

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2022-GBL-EN-100362-v4