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# Increased throughput for IgG quantification using ValitaTiter 384-well plate

## Introduction

ValitaCell's ValitaTiter assay range are rapid, high throughput IgG quantification assays which use fluorescence polarization (FP) for detection. The assays rely on interactions between a fluorescently labeled IgG Fc-specific probe and the Fc of an IgG. FP effectively analyses 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 ~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).
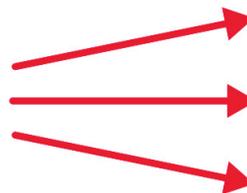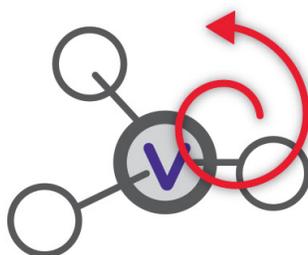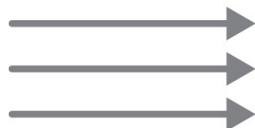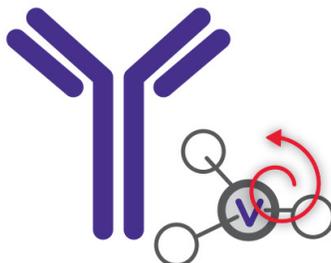


**Figure 1.** ValitaTiter assay principle. Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

## Product Features

The ValitaTiter 384-well assay represents an expansion of ValitaCell's pioneering Quantum technology platform and the ValitaTiter product 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 ValitaTiter 96-well assay. The 384-well format, alongside 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 ValitaTiter 384-well plate can deliver precise, homogeneous measurement of IgG from 2.5 – 100 mg/L in less than 15 minutes. An additional ValitaTiter Plus 384-well product (VAL014) is also available with an extended concentration range (100 – 2000 mg/L).

## Materials and Method

### Materials

- ValitaTiter 384-well plate, catalogue number: VAL013, detection range: 2.5 - 100 mg/L
- CD CHO medium (GibcoTM, 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

- 40 µL of cell culture media was added to each well to reconstitute the Fc-specific probe (pre-dried onto the surface of the ValitaTiter 384-well assay plate);
- 40 µL of each standard/sample was then added into appropriate wells of the 384-well plate;
- A multichannel pipette was used to mix each well 3 times prior to a 5-minute incubation in the dark;
- Post incubation, the plate was measured using FP.
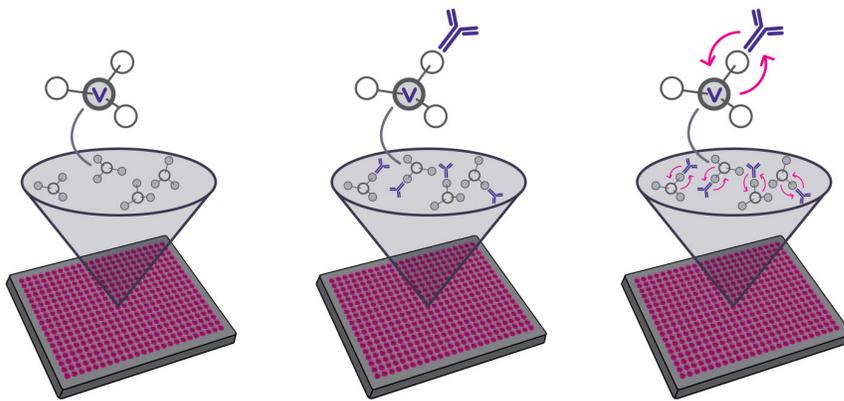
1. ValitaTiter plate is coated with an IgG specific binding peptide
2. IgG in test sample forms complex with the binding peptide
3. IgG concentration is measured using FP on a plate reader

**Figure 2.** Assay Schematic of ValitaTiter assay for IgG quantification using fluorescence polarization.
1. Each well of the plate is pre-coated with a fluorescently labelled IgG Fcspecific probe
2. An IgG sample binds to the probe
3. Binding is measured using fluorescence polarization).

## Results

The performance of ValitaTiter 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 ValitaTiter 384-well plate. Alongside standard curve generation, samples of known concentration were interpolated against the standard curve and accuracy bias assessed.
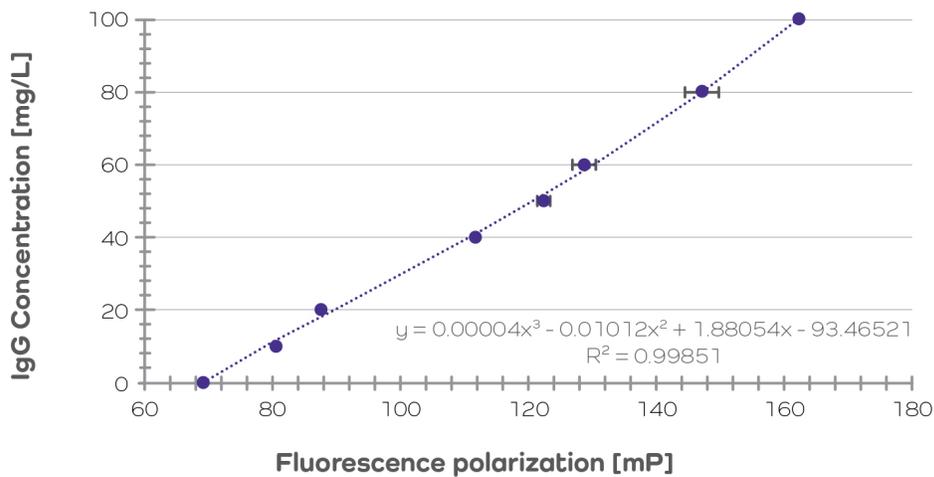


$$y = 0.00004x^3 - 0.01012x^2 + 1.88054x - 93.46521$$
$$R^2 = 0.99851$$

**Figure 3.** ValitaTiter standard curve plotted using a Cubic fit (poly-3 fit) (R2= 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 co-efficient of variation [CV] of </= 2% [Table 2]. Figure 4 demonstrates that the predicted concentration of IgG test samples generated using the ValitaTiter 384-well assay correlates well with the absolute values, with an R2 < 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 ValitaTiter 384-well plate product for quantifying IgG test samples of known concentration.
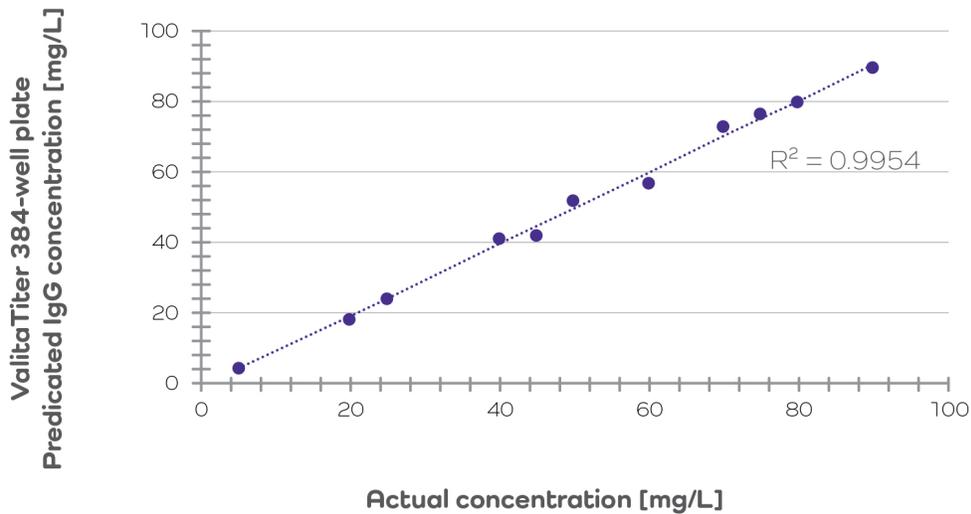


Figure 4. The ValitaTiter assay predicted IgG concentrations plotted against the concentrations for canine polyclonal IgG (R2 = 0.9997).

## Conclusions

The data presented here demonstrates that the ValitaTiter 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 ValitaTiter 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.reduced hands-on time and number of touchpoints.

## Abbreviations

**FP**  Fluorescence polarization
**mP**  Millipolarization units
**IgG**  Immunoglobulin G

## About the Authors

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