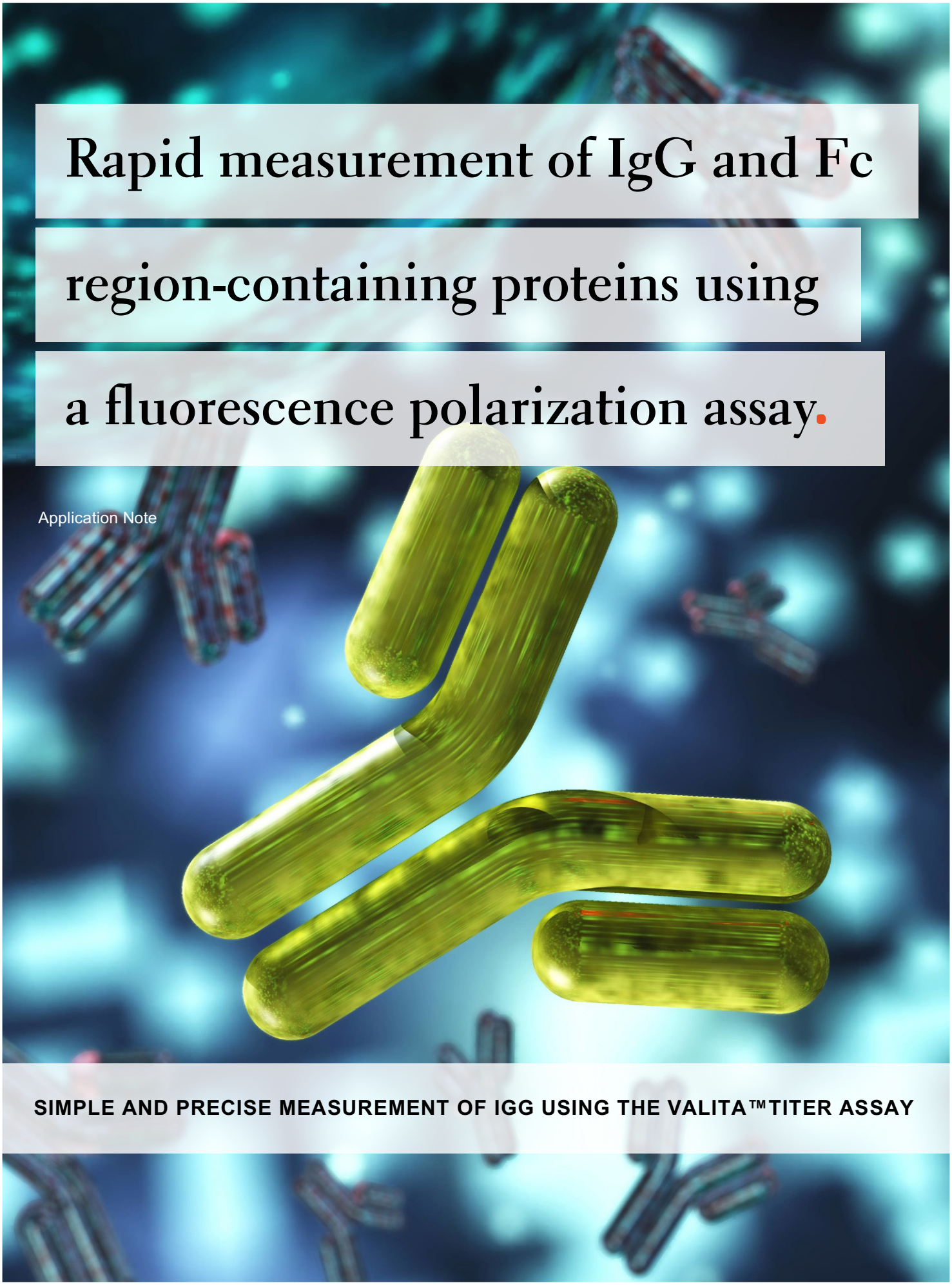
Rapid measurement of IgG and Fc region-containing proteins using a fluorescence polarization assay.

Application Note



SIMPLE AND PRECISE MEASUREMENT OF IGG USING THE VALITA™TITER ASSAY

INTRODUCTION

Monoclonal antibodies are becoming increasingly popular in biopharmaceuticals, requiring vast numbers of samples to be screened during the development of each potential therapeutic. This requires accurate, rapid and high throughput methods for the quantification of IgG and Fc region-containing derivatives. Current workflows for IgG/Fc region quantification – including HPLC, surface interferometry, ELISA and TR-FRET – have a number of drawbacks, such as high costs, labor-intensive methods and long analysis times.

The novel ValitaTITER assay provides a very accurate and cost-effective solution for the detection and quantification of IgG and Fc-containing proteins directly in cell culture supernatants. The assay relies on the detection of IgG and Fc interactions with Protein G by measurement of fluorescence polarization, with a linear detection range from 2.5 to 100 mg/L. The assay is supplied in a 96-well format, providing simple, high-throughput, rapid and fully automatable processing, with low sample volume requirements and no complex preparation steps.
In this application note, we show the optimal protocol for performing IgG/Fc-protein quantification using ValitaTITER assay kits with the Spark® and the Infinite® 200 PRO multimode plate readers.

The Spark multimode reader is Tecan's versatile high-end detection instrument, offering a variety of individual configurations. With its modular and fully upgradeable design, it can effortlessly handle routine and complex assays. Spark's unique Fusion Optics is the first optical solution that combines the flexibility of monochromators with the sensitivity of filter-based detection within a single instrument. This set-up ensures a high degree of versatility for assay development and optimization, paired with exceptional sensitivity for fluorescence-based applications, even in the low signal range.

The Infinite 200 PRO is an easy-to-use multimode plate reader platform that offers affordable, high performance detection for the most common measurement modes, such as absorbance, fluorescence and luminescence. The platform has been continually engineered for excellence, and has evolved into a budget-friendly reader family consisting of six application-focused configurations. The Infinite 200 PRO which was used for testing the ValitaTITER assay kits corresponds to the Infinite F Plex configuration of the reader.

ASSAY PRINCIPLE

The ValitaTITER assay is a rapid, high-throughput assay which relies on the detection of IgG - Fc interactions with an IgG-binding peptide using fluorescence polarization (FP) as a readout mode.

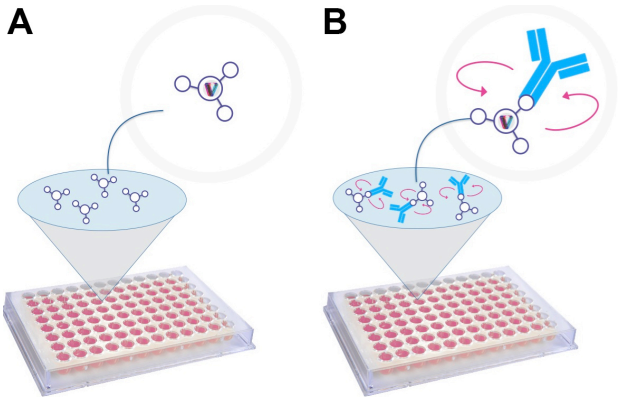


Figure 1: Assay schematic of ValitaTITER assay for IgG quantification using fluorescence polarization. (A) Each well of the plate is coated with a fluorescently-labelled IgG-binding peptide. (B) An IgG sample binds to the peptide, and binding is measured via fluorescence polarization and rotational diffusion.

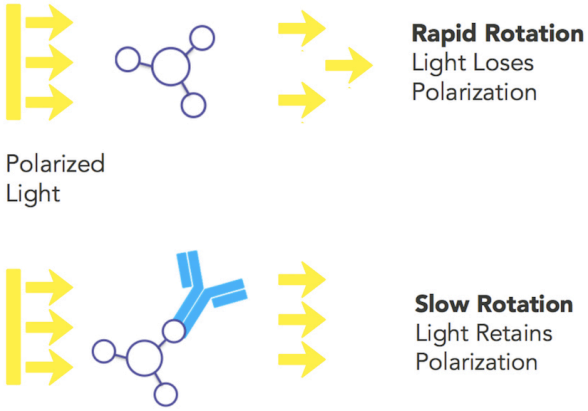


Figure 2: The assay uses fluorescence polarization to quantify IgG. Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

FP effectively analyzes changes in the size of molecules. When a fluorescently-labelled molecule is excited with plane polarized light, light is emitted in the same polarized plane, provided that the molecule remains stationary throughout the excited state. In contrast, if the molecule rotates and tumbles out of this plane during the excited state, light is emitted in a different plane from the excitation light. Small molecules tumble faster in solution than larger molecules, allowing changes in the size of molecules associated with a fluorophore to be measured using the degree of light depolarization.

In this case, unbound, fluorescently-labelled IgG-binding peptide tumbles rapidly and depolarizes the light more than when it is bound to IgG (which is five times larger). FP is expressed as the normalized difference between the intensity of light emitted in the parallel plane (polarized proportion) and the perpendicular plane (depolarized portion) to the polarized excitation light source, which is typically expressed in millipolarization units (mP).

MATERIALS AND METHODS

- ValitaTITER assay kit
- ValitaAPP software
- IgG standard (from human serum)
- Spark multimode reader
- Infinite 200 PRO multimode reader (F Plex configuration)

Experimental procedure

Samples were prepared according to the respective product instruction for use. An eight-point IgG standard curve was prepared as per ValitaTITER assay kit instructions. Briefly, 60 µl of ValitaMab reconstitution buffer were pipetted into each well of the ValitaTITER plate, along with 60 µl of the prepared standards. Contents were mixed and incubated in the dark for 30 minutes before being measured on the Spark and Infinite 200 PRO plate readers, using protocols preconfigured in Tecan software (SparkControl™ and i-control™ respectively), with the measurement settings summarized in Table 1. Raw data was then exported and analyzed using the ValitaAPP software.

Setting	Infinite 200 PRO	Spark (filter optics)	Spark (monochromator)
Measurement mode	Fluorescence Polarization		
Excitation	485 (20) nm	485 (20) nm	485 nm (adjustable bandwidth)
Emission	535 (25) nm	535 (25) nm	535 nm (adjustable bandwidth)
Gain	Optimal	Optimal	Optimal
Mirror	Automatic	Automatic	Automatic
Flash number	Adjustable	Adjustable	Adjustable
G-factor	Calibrate prior to measurement	Calibrate prior to measurement	Calibrate prior to measurement
Lag time	0 µs (default)	0 µs (default)	0 µs (default)
Integration time	40 µs (default)	40 µs (default)	40 µs (default)
Z position	N/A	Calculated from any filled well	Calculated from any filled well

Table 1: Instrument settings for the ValitaTITER assay readout on the Infinite 200 PRO and Spark reader (filter and monochromator optics)

The G-factor compensates for differences in response of the optical components to the parallel and perpendicular polarized light. The G-factor calculation is an important consideration for fluorescence polarization measurements. Although it can be set manually, it is recommended to perform a so-called G-factor calibration for the specific fluorophore used in an FP assay prior to beginning sample measurements.

The G-factor is wavelength-dependent and requires at least one well containing the fluorophore (reference) used in the assay, and one well containing just the buffer solution without any fluorophore in it (reference blank). Once the G-factor has been calculated for a specific fluorophore, this value can be used for all further measurements using the same fluorophore.

RESULTS

An eight-point standard curve (from 2.5 to 100 mg/L) was quantified with the ValitaTITER assay on both readers, the Spark and Infinite 200 PRO, demonstrating a low variation between replicates.

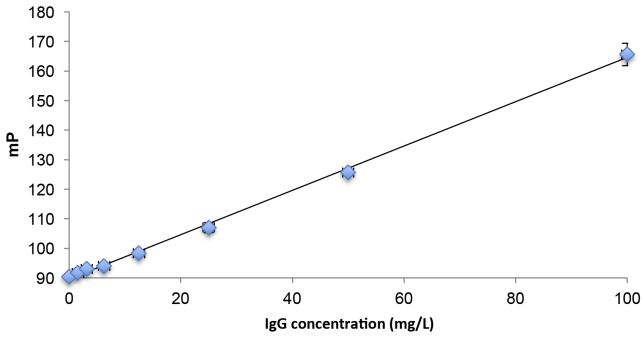


Figure 3: Eight-point standard curve for IgG quantified with the ValitaTITER assay on the Spark microplate reader. Error bars show standard deviation of triplicates.

The performance of the ValitaTITER assay was then compared to HPLC, which is widely regarded as the gold standard method (Figure 4). The correlation of both HPLC and ValitaTITER revealed similar precisions for both methods, with the ValitaTITER being much faster, higher throughput and simpler to perform, due to its homogenous format without the need for complex sample preparation.

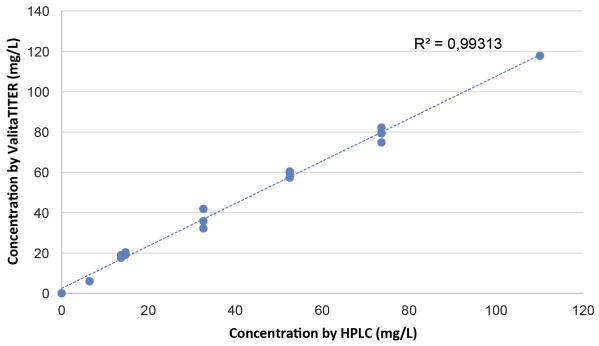


Figure 4: Comparison of IgG quantification with ValitaTITER assay and by HPLC.

CONCLUSIONS

The results demonstrate the suitability of the Spark and Infinite 200 PRO readers for performance of the ValitaTITER fluorescence polarization-based assay for high-throughput titer measurement of IgG and Fc-containing derivatives. The Spark and Infinite 200 PRO exhibited excellent assay performance, providing a high throughput, simple and cost-effective solution for quantification of IgG and Fc-containing proteins. Moreover, the assay can be performed directly in cell culture supernatants, eliminating the need for complex sample preparation steps.

ABBREVIATIONS

FP	fluorescence polarization
HPLC	high performance liquid chromatography
IgG	immunoglobulin G
mP	millipolarization units
TR-FRET	time-resolved fluorescence resonance energy transfer.

About the authors

Dr. Carolanne Doherty is Marketing and Sales Manager at Valitacell. She studied Molecular Biology at University College Dublin and then focused on Systems Biology research during her PhD. Valitacell is a growing biotech company developing innovate technologies for the cell line development sector of bio-manufacturing sciences.

Dr. Katrin Flatscher is an application scientist at Tecan Austria. She studied molecular biology at the University of Salzburg and focused on cell biology and immunology research during her PhD. She joined Tecan in 2007 and has been involved in the development of the Infinite as well as the Spark multimode reader series.

For research use only.

Australia +61 3 9647 4100 **Austria** +43 62 46 89 330 **Belgium** +32 15 42 13 19 **China** +86 21 2206 3206 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170
Italy +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Nordic** +46 8 750 39 40 **Singapore** +65 644 41 886 **Spain** +34 93 595 25 31
Switzerland +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 81 11

400686 V1.0 02-2018

www.tecan.com