Optimized workflow for multiplexing PrestoBlue® & Valita®TITER from ChemStress® plate

TECHNICAL NOTE

Measuring Cell Viability & Cell productivity from the same ChemStress® plate

valitacell™

1. Introduction

A stable *in vitro* environment is crucial for biomanufacturing. However, biological variation remains a major issue. Therefore, improved process controls monitoring cell functionality are necessary.
ChemStress® Fingerprinting is a novel platform technology which allows cell profiling using a panel of specific, small molecule chemical stressors. This is an information-rich technology supplying data on the functional quality of cell culture media environments, clone variants, gene-edited clones and other conditions of interest.

In this technical note, we present data supporting an optimized workflow to multiplex PrestoBlue® [cell viability] and Valita®TITER assays [productivity] from the same ChemStress® plate.

2. ChemStress® technology principle

ChemStress® technology is based on the principle that cells grown in the presence of small-molecule stressors will generate a unique biological phenotype because of their individual response to each stressor. These small molecules have been selected to mimic various stressors that cells are likely to experience in a bioreactor environment during production.
ChemStress® consists of a 96-well plate, with each well coated with a specific chemical to induce different types of cellular stresses. When the cells are incubated with the chemicals their cellular functions are stimulated to varying extents, thereby inducing unique functional responses that we define as cellular fingerprints. These responses can be quantified measuring user-defined assays of interest (e.g. cell death, cellular metabolism, host cell proteins etc.). Determining cellular viability and productivity has been optimised for Chinese Hamster Ovary (CHO) cells using the PrestoBlue® and Valita®TITER assays.

CHO cells are seeded into a ChemStress® plate at a density of 0.2 x 10^6 cells per mL in 90 µl/well (18,000 cells/well) and cultured for 3 days. After exposure to the chemical stressors, cell viability and productivity (based on IgG titer) are measured. These parameters are measured on a microtiter plate reader detecting fluorescence polarization, and analyzed using Valitacell's software, 'Valita®App'. Proprietary algorithms generate ChemStress® fingerprints which are unique to each individual condition.

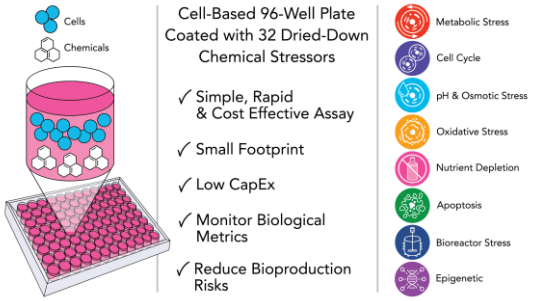

Figure 1. Overview of ChemStress® technology. ChemStress® employs a 96-well plate proprietary format, where each well is coated with a chemical compound selected based on its published ability to simulate specific conditions, or effect specific cellular pathways.

3. Optimized workflow for multiplexing PrestoBlue® and Valita®TITER

Previously, end-point measurements of cell viability (PrestoBlue®) and productivity (Valita®TITER) were performed in parallel using two separate ChemStress® plates. This is due to a partial spectral overlap between the fluorescent label on the IgG-specific probe used in Valita®TITER and the fluorescent probe present in PrestoBlue® assay.
In figure 2, the partial spectral overlap occurring between the Valita®TITER Fluorescein Isothiocyanate (FITC) probe, and PrestoBlue® fluorescent probe resorufin is shown.

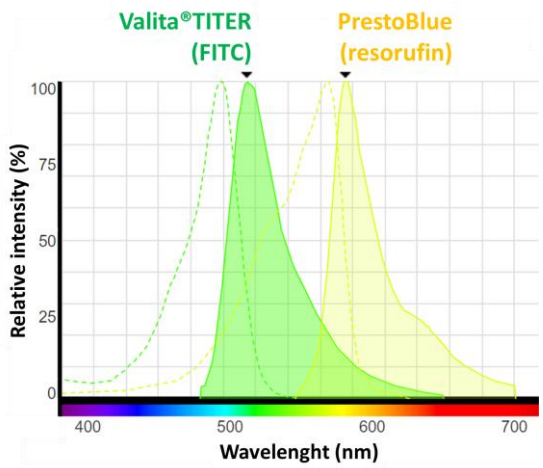

Figure 2. Fluorescent spectra of Valita®TITER: Fluorescein isothiocyanate (FITC) probe and PrestoBlue® probe resorufin. Readapted from Fluorescence Spectra Viewer by Thermo Fisher Scientific.

The data presented here indicates that the PrestoBlue® fluorophore does not affect Valita®TITER measurement interpretation. Thus, titer measurement after PrestoBlue® measurement and in the presence of PrestoBlue® reagent is possible.

This optimized worlfow allows end users to measure both cell viability and titer from the same ChemStress® plates. This reduces potential biological variations arising from parallel experiments with two ChemStress® plates. Morever, the workflow is optimized to reduce the experimental workload, number of cells and ChemStress® plates necessary.

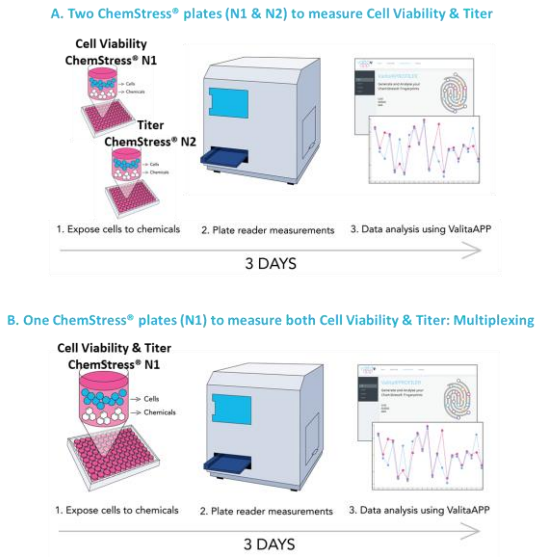

Figure 3. *See next column for figure capture.*

Figure 3. Overview of the original and optimized workflows for viability and titer measurements with ChemStress®.
(A). Original workflow. Two ChemStress® plates were required: N1 to measure Cell Viability and N2 for Titer.
(B). Optimized multiplexed workflow. One ChemStress® (N1) is required to measure both Cell Viability and Titer.
Step 1. Cells are seeded into ChemStress® plate(s) and exposed to chemicals in a static incubator at 37ºC/5% CO_2. Step 2. Cell viability and titer are measured from the ChemStress® plate using PrestoBlue® and Valita®TITER, respectively, on a plate reader. Step 3. Output data is then imported into the Valita®App where ChemStress® Fingerprints are generated.

4. Results

PrestoBlue® fluorescence intensity and Valita®TITER analysis using Fluorescence Polarization detection

PrestoBlue® fluorescence intensity was measured from a standard 96-well plate which contained negative control wells [no cells – empty wells] and wells in which an increasing number of non-producing CHO-K1 cells were seeded [9,000, 18,000 and 36,000 cells/well]. Viable cells maintain a reducing environment within their cytosol. PrestoBlue® uses the reducing power of living cells to quantitatively measure cell viability. PrestoBlue® contains a cell-permeant compound that is virtually non-fluorescent [resazurin]. In the presence of cells, the PrestoBlue® reagent is reduced by the reducing environment of the viable cell and becomes a highly fluorescent dye [resorufin]. As such, fluorescence intensity is proportional to the number of viable cells as demonstrated in Figure 4, where the fluorescence signal increases with an increasing number of CHO seeded cells.

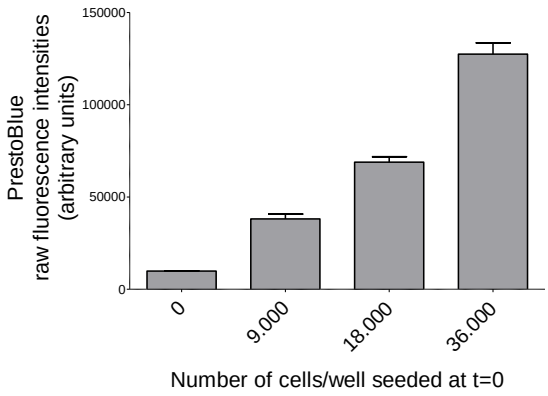

Figure 4. *See next page for figure capture.*

Figure 4. PrestoBlue® fluorescence intensity. After 24 hours, PrestoBlue® raw fluorescence intensities were quantified using a plate reader from a standard 96-well plate where CHO-K1 cells were seeded at densities of 9,000, 18,000 and 36,000 cells/well. A negative control condition [empty well] was also present.

Valita®TITER measurement of IgG in a sample is based on the interaction between a fluorescently labelled [FITC] IgG Fc-specific probe with the Fc of an IgG using fluorescence polarisation [FP] for detection. Because of the fluorescent spectra overlap between FITC and resorufin, investigating the impact of resorufin fluorescent signal on the performance of the Valita®TITER assay was necessary. This was carried out by preparing and analyzing a human IgG standard curve using Valita®TITER, in the presence and absence of Presto Blue® reagent, and an increasing number of cells/well, using a Valita®TITER plate from the same cells used for viability assessment [Figure 4].

Figure 5 shows the output data from human IgG standard curve (0, 6.25, 12.5, 24, 50, 100 [mg/L]) samples analysed using Valita®TITER, in the presence of an increasing number of non-producing CHO-K1 cells/well [9,000, 18,000 and 36,000 cells/well], using FP for detection.
With this controlled experimental setup, we looked at the performance of the Valita®TITER assay to accurately quantify IgG in the presence (Figure 5A) and in the absence (Figure 5B) of PrestoBlue® reagent. The performance outcome of the assay was determined by comparing the raw FP output values obtained for each different IgG concentration with and without PrestoBlue®.
While PrestoBlue® fluorescence (Figure 4), increases proportionally to the viable cell number, it does not influence the performance of the Valita®TITER assay for IgG quantification (Figure 5A). No significant difference was noted between output data [raw FP] from the standard curves analysed in the presence of PrestoBlue® with an increasing number of viable cells versus no PrestoBlue®.
Briefly, this can be explained by the fact that FP is calculated by dividing parallel and perpendicular (with respect to the plane of linearly polarized excited light) fluorescence intensities. Because both the parallel and perpendicular RAW fluorescence intensities increase proportionally to the number of cells and in the same way, therefore, the ratio is constant.

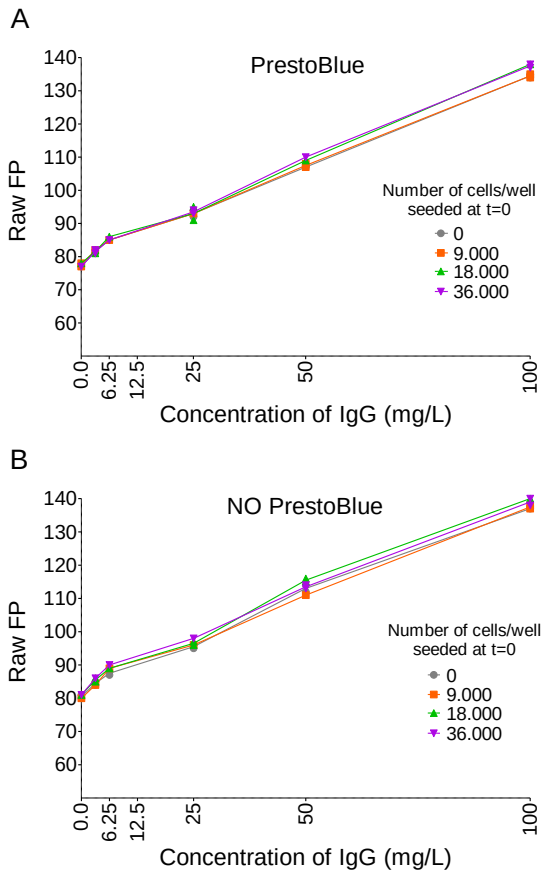

Figure 5. Quantification of FITC FP to measure IgG using Valita®TITER in the presence and absence of PrestoBlue®. Raw FP (typically in millipolarization units (mP)) is plotted against IgG concentration (mg/L) and measured in wells where an increasing number of cells were present in the wells **(A)** in the presence of PrestoBlue® reagent and **(B)** in the absence of PrestoBlue® reagent.

Figure 6 shows the same output data of figure 5. However, in Figure 6 data was plotted without differentiating curves based on cell number but focusing only on the effect of PrestoBlue® on the performance of Valita®TITER for IgG quantification. Here we observed a 4% reduction in FP due to the presence of PrestoBlue®.

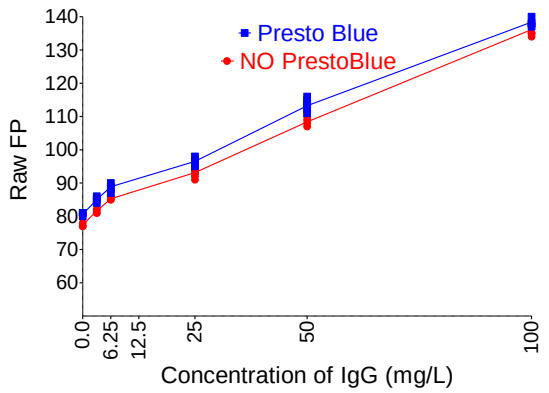

Figure 6. PrestoBlue®-induced reduction of FITC FP using Valita®TITER in the presence of PrestoBlue® Raw FP (typically in millipolarization units (mP)) is plotted against IgG concentration (mg/L) with the same data shown in Figure 5 produced with different cell number, plotted here without differentiating curves based on cell number but focusing only on the presence and absence of PrestoBlue® reagent.

5. Conclusion

The results presented in this technical note demonstrate that PrestoBlue® reagent induces a minor reduction (4%) in FP due to the fluorescent spectra overlap between FITC [label on the Fc-specific probe utilised in Valita®TITER] and resorufin. Most importantly, this PrestoBlue®-induced FP reduction is not influenced by the magnitude of the PrestoBlue® fluorescence intensity proportional to the number of cells. Therefore, PrestoBlue® fluorophore does not affect Valita®TITER measurement interpratation.

In conclusion, titer measurement, using Valita®TITER and FP for detection, of the same cell samples, previously analysed for viability using PrestoBlue® is possible.

This optimized worlfow allows the measurement of both cell viability and titer from the same ChemStress® plate. This reduces any potential biological variation arising from running experiments in parallel in two ChemStress® plates. Morever, this optimised workflow reduces the experimental workload and cost, as less plates, reagents and cells are needed.

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