Multiplexed cell- and bead-based assays in a no-wash format for biologics screening and characterization

David Onley, Lucy Chammas, Diana Caracino and Paul G. Wylie

TTP Labtech Inc, One Kendall Square, Suite B2303, Cambridge MA 02139-1594, USA

introduction

Biologics such as antibodies are ideally suited for therapeutic use against cell-surface disease targets. When screening, the use of cells expressing native target proteins minimises the risk of false positive binding (which can occur when using recombinant proteins) leading to improved hit quality.

Traditional screening methods to identify and characterise hits often report a single measurement per well, due to either assay or instrumentation constraints. Multiplexed assay formats facilitate the reporting of multiple readouts to further improve data quality and enhance productivity, whilst also conserving sample.

Here we present the results generated from multiplexed no-wash protocols using TTP Labtech's mirrorball, a laser scanning cytometer. To model antibody screening, anti-EGFR antibodies were titrated against multiplexed target-expressing and control cells to determine specific versus non-specific binding in one productive screen. Additionally, we report the results of a no wash, IL-6 and IL-8 cytokine detection assay where reagents from two commercially available single-plex ELISA kits were transferred onto TTP Labtech sol-R™ coded beads and multiplexed together. The results of this alternative fluorescence-based approach demonstrate similar performance to ELISA, but with a significantly streamlined work-flow.

Overall, the data presented here show the versatile nature of and advantages of multiplexing assays using TTP Labtech's mirrorball for improving the productivity of biologics discovery.

1. multiplexed cell-based antibody binding assay

The EGF receptor is known to play a role in certain pathological conditions including breast cancer and has been a target of interest for the development of therapeutic molecules such as herceptin. A431 cells express large numbers of EGF receptors whereas Jurkat cells have no detectable EGF receptors. Using these cell lines and commercially available reagents we can model an antibody screening campaign in singleplex and multiplex using the mirrorball fluorescence cytometer. Positive and negative cells were coded by labeling with different concentrations of Celltrace™ Far Red DDAO-SE (ThermoFisher Scientific). Cell types were identified based on their fluorescence intensity in the red channel and the binding of antibody to each cell type was determined in the green channel.

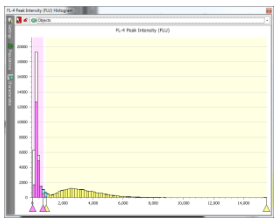

Fig 1. Cell coding. Jurkats (yellow) and A431 cells (pink) were stained with 1000nM and 100nM Celltrace Far Red respectively.

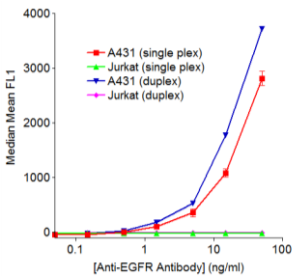

Fig 2. Binding of anti-EGFR antibody in singleplex and multiplex assay format.

2. sol-R beads

ELISA assays currently play a significant role throughout biologics discovery from identification of novel biologic molecules (e.g. antibody binding to target antigen), functional characterization (e.g. quantification of cytokine release upon cell treatment) and beyond. Although such assays results are powerful, ELISA protocols require multiple wash steps and are not amenable to multiplexing or high-throughput.

sol-R color-coded beads are 8.5µm in diameter, with consistent binding capacity for all 5 codes and have been developed for use on TTP Labtech's mirrorball instrument. The transfer of tests onto sol-R beads, facilitates conversion to no-wash assay format and multiplexing. These dual advantages offer significant process improvements.

3. conversion of ELISA to fluorescence-based ELISA format

To demonstrate the advantages of converting from ELISA to fluorescence-based ELISA (fluorescent linked immunosorbant assay), reagents from two commercial ELISA kits were transferred onto sol-R beads (IL-6 and IL-8; R&D Systems #DY206 and #DY208 respectively).

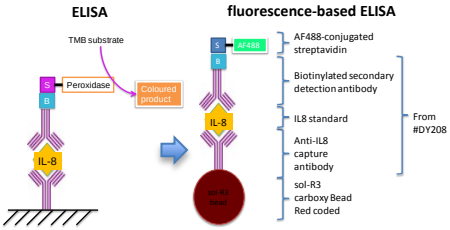

Fig 3. Conversion of ELISA to fluorescence-based ELISA. In fluorescence-based ELISA, the capture antibody is immobilized onto carboxy-coated sol-R3 beads and binding is detected using Alexa Fluor 488-conjugated streptavidin. In this way, the components of the binding sandwich are directly transferred over onto the bead.

4. no-wash fluorescence-based ELISA offers significant process improvement over ELISA

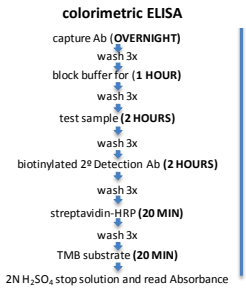

Fig 4. The fluorescence-based ELISA assay protocol is much simpler than ELISA and is very automation-friendly.

5. no-wash fluorescence-based ELISA gives similar performance to ELISA

For both IL6 and IL8 assays, the conversion from multistep, multiwash ELISA to a single step no-wash fluorescence-based ELISA yielded similar assay performance.

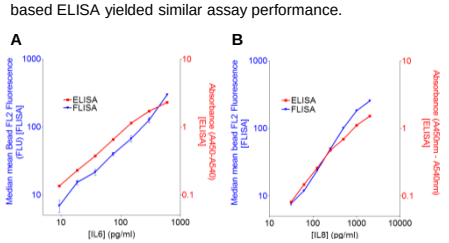

Fig 5. No-wash fluorescence-based ELISA produces similar assay performance to ELISA for both IL6 (A) and IL8 (B) cytokine quantification.

6. multiplex fluorescence-based ELISA produces the same results as singleplex fluorescence-based ELISA

Converting a single assay from a multiwash to a no-wash assay already offers a significant process improvement. However, the ability to immobilize different capture antibodies to the sol-R beads allows the possibility of combining multiple tests within the same well. In this example, three different intensity sol-R beads were coated with either anti-IL-6, anti-IL-8 or no capture antibody.

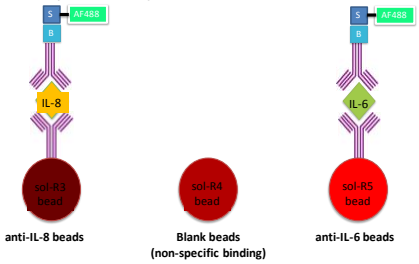

Fig 6. Multiplex color-coding IL-6, IL-8 and blank beads

All three bead types and detection reagents were combined in the same well. Having already shown that a singleplex fluorescence-based ELISA had comparable performance to ELISA, it was important to see whether carrying out cytokine assays in multiplex fluorescence-based ELISA affected the assay performance. Data below shows specific binding responses in singleplex assays with similar results to the corresponding singleplex assay.

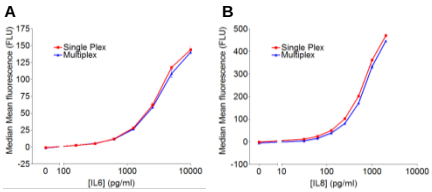

Fig 7. Similar assay performance for quantification of IL-6 (A) and IL-8 (B) in both singleplex and multiplex fluorescence-based ELISA.

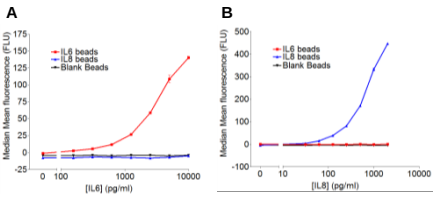

Fig 8. Only the correct beads detect IL-6 (A) or IL-8 (B) cytokines in multiplex fluorescence-based ELISA assay format. There is no binding to the sol-R4 blank beads.

conclusions

Data presented here demonstrate the power of a multiplexed assay approach for screening and hit characterization assays using both cell- and bead-based methods. mirrorball and sol-R beads enable:

- biologics screening with cells expressing targets in their native conformation
- combination of target and parental cells in the same well for direct cell line binding comparison
- conversion of multistep, multiwash ELISA to single-step no-wash fluorescence-based ELISA assay with similar assay performance
- combined multiple cytokine measurements into a multiplex assay format
- significant process and workflow advantages offered by a no-wash assay format and the ability to multiplex tests
- time to be freed up for other valuable tasks

www.ttplabtech.com

TTP Labtech Ltd
Melbourn Science Park, Melbourn
Hertfordshire, SG8 6EE,
United Kingdom
Tel: +44 (0) 1763 262 26
Fax: +44 (0) 1763 261 964

TTP Labtech Inc
One Kendall Square, Suite B2303
Cambridge MA 02139-1594,
United States
Tel: +1 (617) 49 9794
Fax: +1 (617) 4949795

TTP Labtech India Private Limited
SRS Tower – 212, 2nd Floor
Sector 31, 14/5 Mathura Road
Faridabad (NC Delhi) 121003, India
Tel: +91 9910397385
+91 9968291292