mirrorball®
application note

improving the reliability of cell-based immunoassay screening through multiplexing with mirrorball®

introduction

A significant number of biopharmaceutical groups are looking to develop therapeutic antibodies, antibody-like molecules or fragments against cell surface antigens. To improve the reliability of hits, it is beneficial to screen against cell lines expressing the antigen of interest rather than immobilised purified antigen. The advantage of the cellular approach is that antigen epitopes are more likely to be preserved in their natural confirmation and therefore the incidence of false positive, or negative binding events should decrease. However, when cells die their membranes can become ruptured and expose a large range of proteins, which can lead to significant off-target antibody binding. A further concern is that the target protein may undergo degradation during apoptosis, which could lead to lower apparent binding levels through loss of epitope integrity. By simply eliminating dead cells from analysis, the quality of screening data can be further enhanced.

TTP Labtech's mirrorball fluorescence cytometer enables users to gain quality decision making data fast for immunoassay screens through a combination of system and data reliability. The mirrorball design is different from flow cytometry and ELISA: it uses TTP Labtech's laser scanning technology with proprietary background rejection optics to provide HTS-friendly workflows that deliver gold standard data quality for sol-R™ bead-, *in-situ* adherent-, or suspension cell assays. The mirrorball approach centres on delivering robust data by eliminating wash steps from assay immunoassay protocols, carryover between wells, the potential for flow cell clogging, or changes to binding equilibrium when samples are mixed with sheath fluid. Data validation tools within Cellista software offer built-in quality control readouts including total cell count/well and the ability to visually confirm results using mirrorball images for additional confidence in assay results.

In this application note we describe a homogeneous mirrorball antibody screening assay against a membrane associated target, with the ability to discriminate between live and dead cells for enhanced data quality.

immunoassay protocol

cell treatments (target expressing transfected and parental Ba/F3 cells)

- To generate a predominantly dead cell pool, half of the cells (target and parental) were treated with 2 µM staurosporine in cell culture medium overnight.
- To generate a predominantly healthy cell pool, the other half of the cells (target and parental) were treated with cell culture medium only.

procedure

- each of the four cell types (transfected, live or dead; parental, live or dead) was diluted to 125,000 cells/mL in PBS buffer containing 3% BSA. The following detection components were added to each cell suspension:
  a. 800 ng/mL detection antibody (AF 488)
  b. 1 µM propidium iodide (dead cell marker)
  c. 2 µM calcein deep red acetate (live cell marker)
- homogeneous mix-and-read immunoassays were set up in a 384-well assay plate (Corning #3712) with each well comprising:
  a. 10 µL of test antibody (serial dilutions)
  b. 10 µL of cell suspension/detection component mixture
- the plate was incubated at 37 ºC, 5 % CO_2 for 2 hours, then scanned on mirrorball to gain decision making results in just 12 minutes

key benefits

The purpose-built system design of TTP Labtech's mirrorball ensures reliability for

- **gaining decision making datafaster**
- **confidence in the integrity of screening hits**

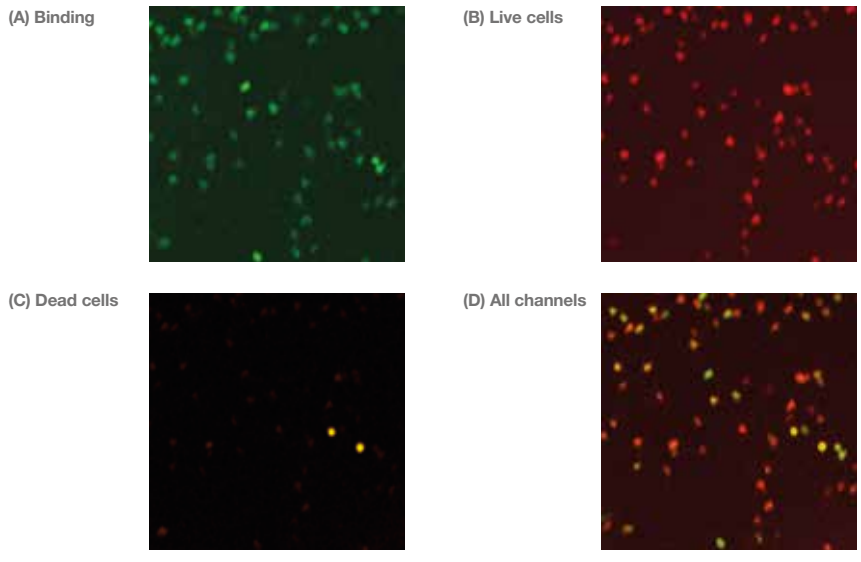

Fig 1. Antibody binding to live and dead target-transfected Ba/F3 cells. Antibody binding was detected by Alexa Fluor 488 labelled detection in the green FL2 channel (A); live cells labelled with calcein deep red acetate were detected in FL4 (B); propidium iodide-stained dead cells were detected in FL3 (C) and all three channels overlaid (D).

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results

In this protocol, the cells were simultaneously excited with the 488nm and 640nm lasers. Data was collected in the green, orange and red channels of mirrorball for Alexa Fluor 488 binding, dead cell labelling (propidium iodide) and live cell labelling (Calcein deep red acetate), respectively (Fig 1). Propidium iodide is a cell impermeant stain that becomes fluorescent upon binding to nucleic acids. Upon cell death, or the late stages of toxicity, the cell membrane loses integrity, which allows propidium iodide to gain access to the cell and fluorescently stain nucleic acids. Calcein deep red acetate can readily pass through the intact cell membrane where it is cleaved by enzymes within live cells. The consequent fluorescence signal was detected in the red FL4 channel.

Using mirrorball's template-driven Cellista software, cells were identified based on size, then sub-classified to identify live or dead cells using simple gating criteria based on the intensity of propidium iodide and calcein deep red acetate staining (Fig 2).

With cell classifications in place, the binding of a test antibody to only live Ba/F3 cells was reported (Fig 3a). The test antibody was observed to bind specifically to live target-transfected cells over live parental cells. In this study, non-specific binding to dead parental cells was not observed. Interestingly, binding to dead transfected cells resulted in a halving of the signal achieved with live transfected cells (Fig 3b), which may be due to degradation of the binding epitope upon cell death. The results of this study show that data accuracy may be improved through the removal of binding to dead cells from analysis.

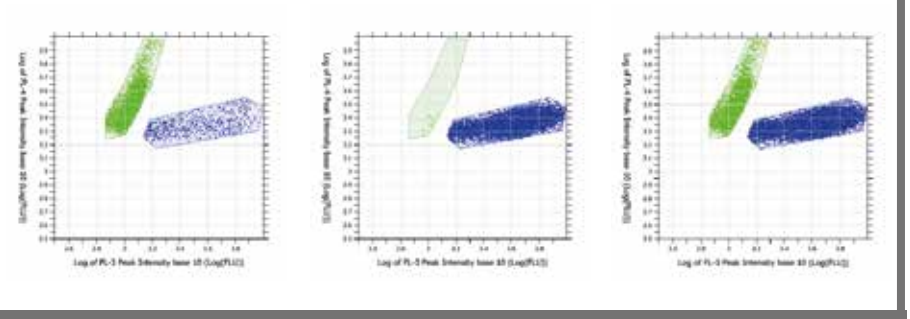

Fig 2. 2D scatter plots for simple live/dead cell gating criteria within the Cellista software. Cell health is determined by the relative propidium iodide (FL3 on the x axis) to calcein deep red staining (FL4 on the y axis) using wells containing untreated cells (A) or staurosporine-treated cells (B). Wells containing a mixture of untreated and staurosporine-treated cells are shown in (C). Live cells are represented in green and dead cells in blue.

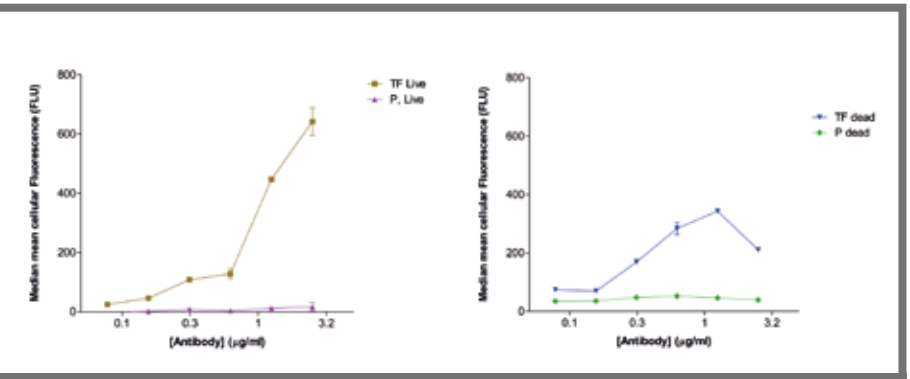

Fig 3. Antibody binding to live (A) or dead (B) Ba/F3 cells. Transformed cells (TF) express the target of interest whereas the parental cells (P) do not express the target protein.

conclusions

Designed for reliability, TTP Labtech's mirrorball ensures high quality data at HTS speeds for confident screening against membrane-associated targets. mirrorball enables:

- selection of the most appropriate cellular model (suspension, or adherent) to suit therapeutic focus and improve the physiological relevance of immunoassay screens
- no-wash, multiplexed immunoassay protocols enhance data robustness and simply workflows compared to cell-based ELISAs
- no fluidic system design to eliminate potential compromises to data integrity and to enable walk away HTS automation
- built-in Cellista quality control tools facilitate the visual confirmation of results for assured decision making

Progress only true hits using TTP Labtech's mirrorball for a more efficient discovery process.

designed for discovery

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