mirrorball®
application note

simple competition binding assays with mirrorball®

introduction

Identification of novel biological therapeutic agents comprises multiple steps from target selection, initial screening to identify lead candidates, affinity maturation and subsequent rounds of lead characterisation and optimisation to improve binding affinity and/or potency. Competition assays are particularly relevant in the latter stages, when identifying variants with higher target binding affinity. One advantage of identifying higher affinity antibodies is these are more likely to have improved clinical efficacy, requiring less frequent patient treatment doses. If the target protein is a cell receptor, common competition assay formats use a labelled form of the endogenous ligand which can then be competed away using the test biologic e.g. antibody. The labelled component is used at a partially saturating concentration, usually at the EC_{50}. No-wash assay formats are advantageous to ensure that binding is at equilibrium when plates are read. In addition, homogeneous assay formats offer a simple and convenient workflow that is amenable to automation and free up FTE time compared to multi-step, multi-wash ELISA or flow cytometry protocols.

TTP Labtech's fluorescence cytometer is neither a flow cytometer, nor an ELISA reader. Instead it uses TTP Labtech's laser scanning fluorescence cytometry to provide HTS-friendly workflows that deliver gold standard data quality. The mirrorball's no-fluidics approach eliminates the potential for carryover between wells, system clogging, or changes in binding equilibrium that occur when samples are mixed with sheath fluid.

The mirrorball design, with its proprietary background rejection optics, facilitates the use of streamlined no-wash assay formats for cell, or bead-based competition assays. This technote describes the protocol for a simple bead-based antibody competition assay using TTP Labtech's sol-R™ coded beads as an example assay format.

method

This method prepares enough beads for 5 x 384 well assay plates. Transfer sol-R4 streptavidin-coated beads (TTP Labtech cat# 4150-09031) into 1.7 mL low binding microfuge tube (Sorensen cat# 27210), this contains 2 x 10^5 beads. Wash 3 x 0.5 mL PBS. Re-suspend in 0.5 mL PBS. Incubate with 10 µg/mL of biotinylated anti-mouse IgG (JIR cat# 115-065-062) for 1 hour with rotation at room temperature in the dark. Wash 3 x 0.5 mL PBS, 1 % BSA. Re-suspend in 0.5 mL PBS, 1 % BSA, 0.05% sodium azide and store at 4°C until required (assuming no losses, these coated beads are now at 4 x 10^5 beads/mL).

Prepare a half log dilution series of unlabelled mouse IgG (JIR cat# 015-000-003) and add 10 µL per well to 384-well assay plate. Prepare a bead detection mixture containing: 5 x 10^4 anti-mouse IgG-coated beads/mL and 200 ng/mL of Alexa Fluor® 488-conjugated mouse IgG (JIR cat# 015-540-003). Add 10 µL per well of bead detection mixture, incubate for 3 hours and scan on mirrorball in just 12 minutes per plate (Fig 1, 2 and 3).

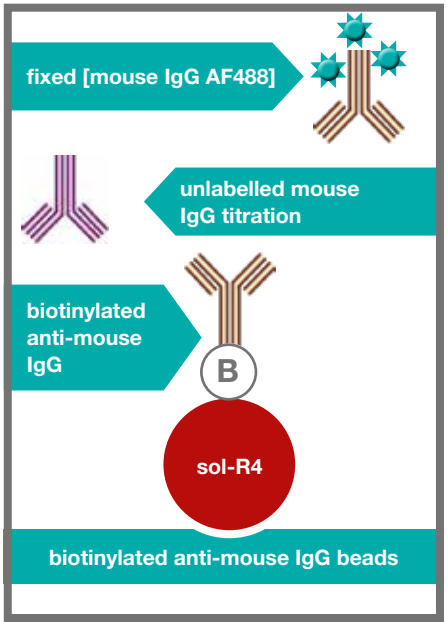

Fig 1. Binding schematics for this model competition assay. Biotinylated anti-mouse IgG is pre-bound to streptavidin-coated TTP Labtech sol-R beads. A titration of unlabelled mouse IgG competes with a fixed concentration of Alexa Fluor 488-labelled mouse IgG.

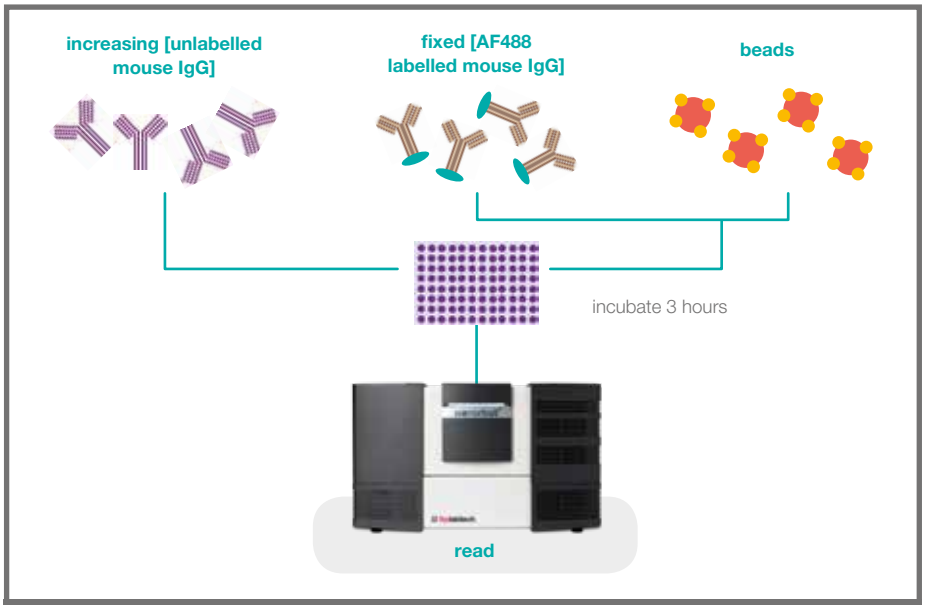

Fig 2. Simple no-wash mix and read assay workflow. Two additions per well only; 10 µL of unlabelled mouse IgG and 10 µL of detection mixture containing pre-coated anti-mouse IgG beads and Alexa Fluor 488-labelled mouse IgG.

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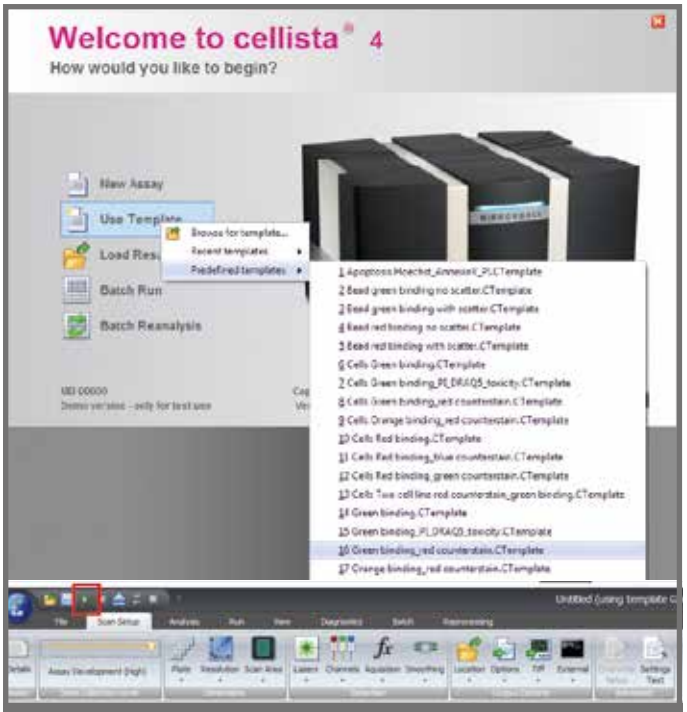

Fig 3. Simple instrument set-up using predefined templates.

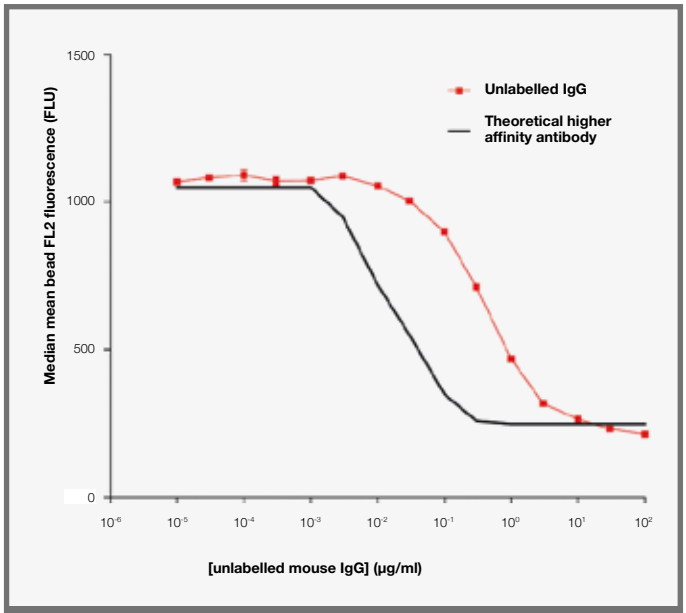

Fig 4. Competition binding data. Red line shows binding data with unlabelled IgG. Black line is a theoretical dataset depicting the performance of an unlabelled IgG with enhanced binding affinity for the target protein immobilised on the beads. Data shown are mean and standard deviation of quadruplicate wells.

results

TTP Labtech's mirrorball identifies beads based on their red intensity and subsequently detects the amount of labelled antibody binding to the bead in the green channel. Figure 4 shows a typical competition binding profile from mirrorball. The red line shows that as the concentration of unlabelled mouse IgG increases, the amount of Alexa Fluor 488 labelled antibody binding to the beads decreases. The black line shows a theoretical curve for an antibody with increased binding affinity to the beads. When comparing multiple related antibodies (which may have single amino acid substitutions in their binding domains) using a competition assay, those candidates where the competition binding curve shifts to the left are most desirable.

In this demonstration, a biotinylated anti-mouse IgG is used as a target, but any other biotinylated substrate can be immobilised onto streptavidin-coated sol-R beads.

Alternatively, a cell line expressing the target protein on the cell surface can be used instead of a coated bead for this purpose. This simple approach may be used to rapidly determine the relative binding affinities of antibody variants as part of the lead candidate selection workflow.

conclusions

Together, TTP Labtech's mirrorball and sol-R coded beads enable researchers to readily carry out no-wash competition assays at equilibrium in order to identify lead biologics candidates with the most appropriate target protein affinities. In addition to bead-based protocols, this approach is also applicable for use with suspension and adherent cells *in-situ*. The mirrorball approach has the following benefits:

- simple no-wash homogeneous assay formats facilitate automation and decrease FTE "hands-on" time, freeing up time for other tasks
- simple instrument set-up: no requirement for specialist operators or daily maintenance routines
- simple no-fluidics design combined with no-wash assay protocols ensure binding equilibrium is maintained during plate reads for data robustness

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