simultaneous determination of antibody binding, specificity and titre on the mirrorball® fluorescence cytometer

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introduction

The antibody discovery process relies on consecutive screens to test the same antibody sample for binding affinity, target specificity and antibody titre.

Conventionally such screens are done by ELISA or flow cytometry, but both techniques have technical limitations that restrict their utility in high-throughput screening environments

- multiple wash steps are required, with significant hands-on time from the operator restricting throughput.
- ELISA assays generate only singleplex readouts.
- sample throughput rate and plate capacity of most flow cytometers is incompatible with screening environments.

Here we present a simple no-wash assay to screen antibody samples for binding to a target cell line, non-specific binding to a control cell line and antibody titre by binding to a bead.

1. assay format

A mixture of EGFR-expressing and EGFR-negative cells, anti-IgG capture beads, detection antibody and antibody sample is added to the same well of a 384-well assay plate.

The two cells lines and beads are stained with different combinations of red and orange fluorescent dye respectively, to enable classification and multiplexing.

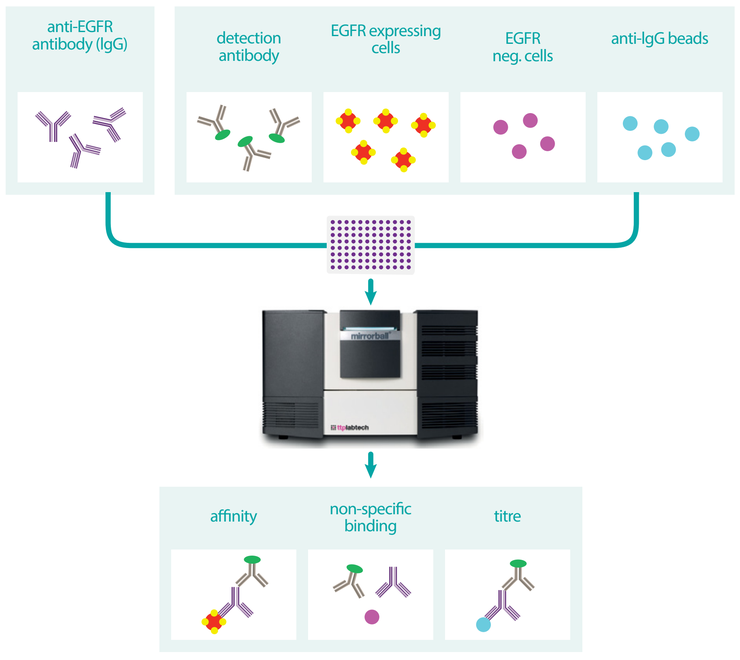

Fig 1. multiplex assay principle

The amount of sample binding to each cell type or bead is determined using a single, green-fluorescent detection antibody directed against the antibody sample. This set-up allows simultaneous determination of target-specific binding, non-specific binding, and antibody titre in a single assay.

2. materials

- sample: mouse anti-EGFR monoclonal antibody
- sol-R™ 5 streptavidin-coated beads
- Bead capture antibody: goat anti-mouse [*biotinylated, F(ab')$_2$ domain specific*]
- A431 cells in suspension (EGFR-positive)
- Jurkat suspension cells (EGFR-negative)
- Cell Tracker Orange CMTMR dye – orange fluorescent counterstain
- DRAQ5 – red fluorescent nuclear dye/counterstain
- Detection antibody: goat anti-mouse (*AF488-conjugated, Fc domain specific)*

3. methods

1. Coat the sol-R beads with the biotinylated anti-mouse IgG capture antibody
2. Stain the Jurkat cells with 5 µM Cell Tracker Orange CMTMR
3. Leave the A431 cells unstained
4. Prepare 4 mL of the detection mixture: Combine 2 x 10^5 cells or coated beads of each type, then add DRAQ5 to 5 µM and detection antibody to 1.6 µg/mL
5. To each well of 384-well assay plate add 10 µL of mouse anti-EGFR antibody sample
6. Add 10 µL/well of detecting mixture to the sample
7. Incubate for 2 hours, then read plate on the mirrorball fluorescence cytometer

4. cell classification

cell type classification:

- Pink: Jurkat cells, counterstained with Cell Tracker Orange and DRAQ5
- Red: A431 cells counterstained with DRAQ5 only.
- Blue: coated sol-R titre beads, intrinsically brighter red fluorescence than either cell type.

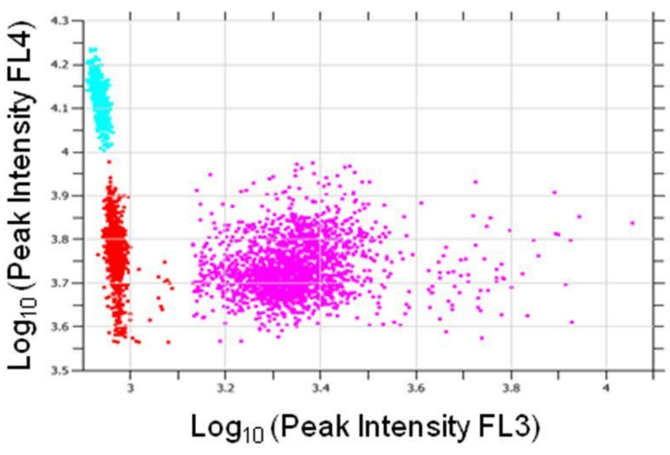

Fig 2. 2D scatter plot of orange (FL-3) versus red (FL-4) fluorescence peak intensity

5. binding affinity

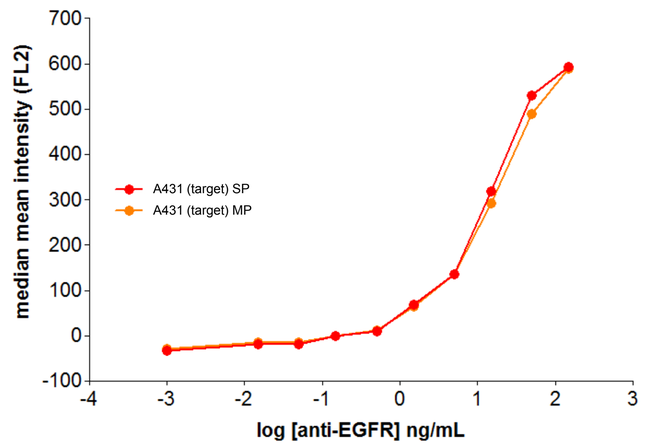

Fig 3. Titration of the mouse anti-EGFR sample against the A431 target cell line. Singleplex (SP) vs. multiplex (MP) assay.

6. binding specificity

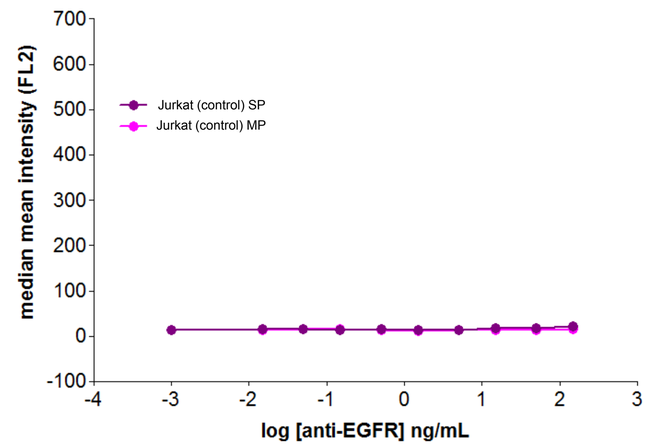

Fig 4 Titration of the mouse anti-EGFR sample against the Jurkat control cell line. Singleplex (SP) vs. multiplex (MP) assay.

7. antibody titre

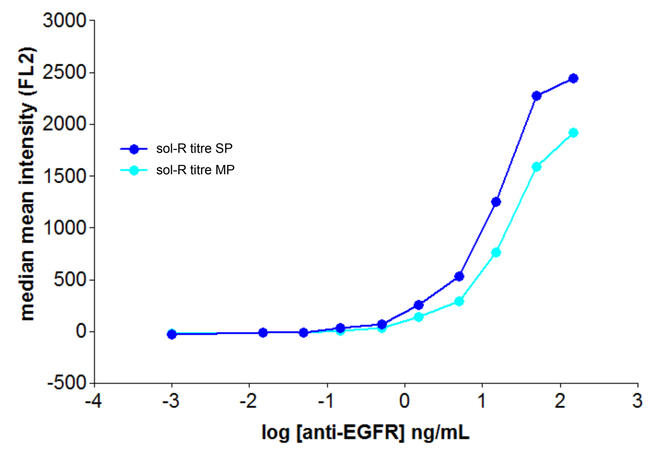

Fig 5 Titration of the mouse anti-EGFR sample against the coated sol-R beads. Singleplex (SP) vs. multiplex (MP) assay

conclusions

The data presented demonstrate a simple binding assay that enables multiplexed antibody screening for affinity, specificity and titre in a single experiment. This provides:

- a homogenous (no-wash) assay format minimising the "hands-on" time for the operator and, is compatible with simple liquid handling dispensers for set-up
- these features make this method ideally placed to accelerate the pace of antibody screening, whilst minimising the costs associated with setting of multiple washed assays