

Application Brief

BIOLOGICS DISCOVERY: ANTIBODY ENGINEERING

A Mix-and-Measure Assay for Screening Hybridoma Supernatants on IntelliCyt Platforms

Key Features & Benefits

FEATURES

- Mix-and-measure
- Color-coded cells combined in each well
- Screen using antigens in their natural conformation in intact living cells

BENEFITS

- Streamlined, shortened assay protocol; reduces cost and variability
- Simultaneous test binding of antibodies to target and control cells; specificity in your primary screen
- Ability to detect conformational epitopes; fewer false negatives

Introduction

Antibody engineering is critical to development of new therapies in many fields such as oncology, where monoclonal antibodies are currently the most widely used immunotherapy for cancer (1). Screening hybridoma libraries for antibodies that bind to cell surface antigens is a time and resource intensive process (2). The current screening workflow often involves several individual testing steps, including different biochemical tests that test for binding, specificity, followed by cell based assays. The first step in the process, testing for binding, is traditionally performed during a primary screen using an ELISA assay in which the target antigen is purified, isolated and immobilized on the surface of the assay plates. Once binders are found in the primary screen, subsequent testing is required to ensure that the antibodies do not cross react with other antigens (specificity testing). Positive antibodies are further tested to determine if they bind to the antigen in its natural environment within the cell membrane using cell based binding assays. In addition to being costly and laborious, purified

protein assays such as ELISA that utilize denatured antigens can generate false negatives - potential missed hits that would have bound to antigens in their native conformation (**Figure 1**). IntelliCyt has developed a “mix-and-measure” intact cell assay for primary screening that simultaneously assesses antigen binding and cross-reactivity. This assay reduces workflow steps and increases productivity. Additionally, this approach reduces the potential for false negatives due to testing with denatured antigens.

Assay Principle

Two cell lines were utilized to simulate a hybridoma screening process and highlight the power of multiplexing different cell types in a single well (**Figure 2**). The target cell line, Jurkat 45.01, expresses CD45, whereas the control cell line, Jurkat D1.1 does not. Both cell lines express CD3. The control cells were labeled with a green fluorescent dye (Calcein AM) while the target cells were left unstained, and both cell types were mixed in equal proportions into a single cell suspension for the assay at a concentra-

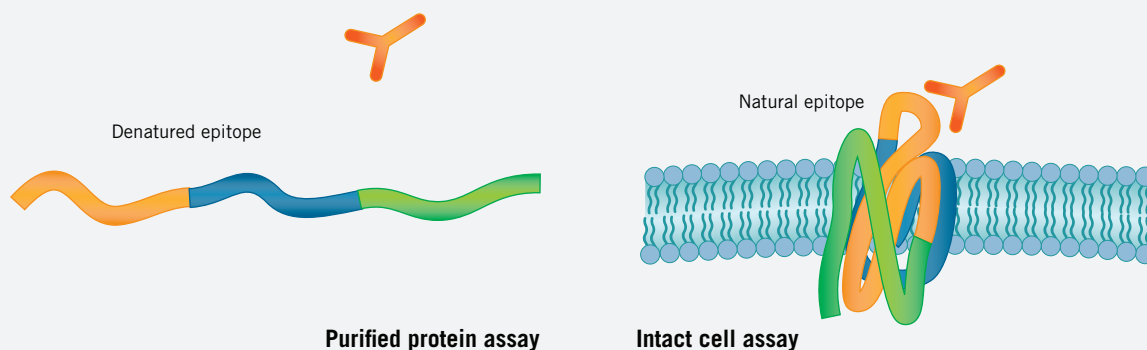


Figure 1. Intact cell based assay allows screening of antibodies to conformational epitopes that may not be available in ELISA and other purified protein assays where the antigens are often unfolded and denatured during the purification process.

tion of 1×10^6 cells/mL. A representative assay plate was set up to mimic the presence of both specific and cross reactive antibodies binding to the cells. Specific binding was shown with an anti-CD45 antibody to represent specific hits, as only target cells expressed this antigen. The ability to detect cross reactive antibodies was demonstrated with wells spiked with an anti-CD3 antibody which binds to both target and control cells. Binding to the control cell signals either cross reactivity or nonspecific binding, essentially providing an internal control for every well of the assay to maximize the success of the screen. This is a mix and read assay in which the primary antibody was added and incubated with the

cells for 30 minutes, followed by the addition of the detection antibody and incubation for a further 30 minutes.

Results & Conclusions

To determine the specificity and detection range of the assay, an anti-CD45 control antibody was tested in dose response curves against the CD45+ and CD45- Jurkat cells. **Figure 3** shows that the antibody specifically bound to the CD45+ cells. A significant signal over background was observed with approximate antibody concentrations between 1 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$.

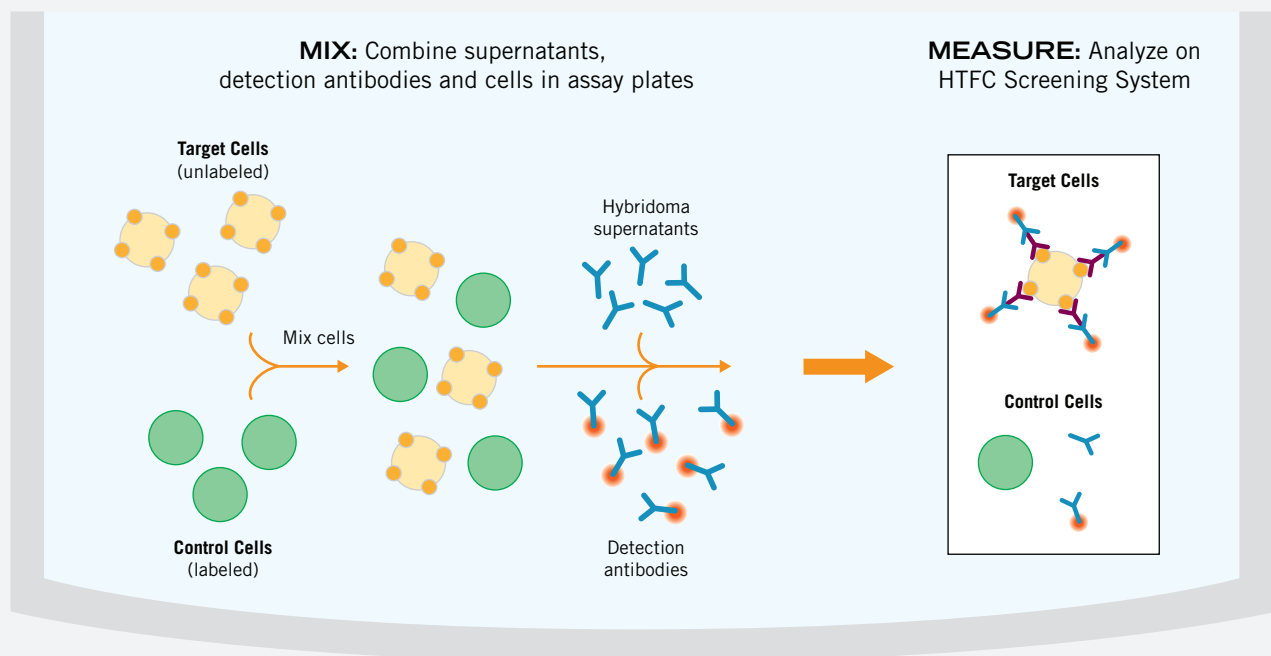


Figure 2. Target cells (unlabeled) and control cells (Calcein labeled) are mixed together, and distributed into all the wells of the assay plates. Test antibodies, such as those present in supernatants from a hybridoma library are added to the assay plates, followed by incubation with a red fluorescent detection antibody. Plates are analyzed on the HTFC Screening System, which allows the detection of binding of antibodies from the hybridoma supernatants to target and control cell populations simultaneously. The assay volume for each well was 20 μL . 1 μL of the test antibodies was added to each well.

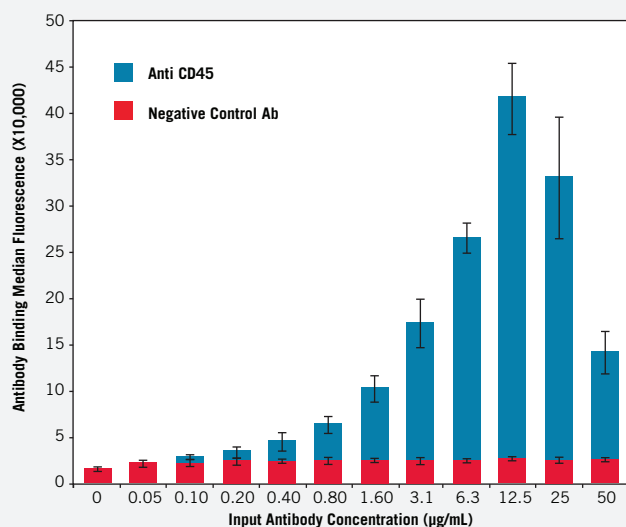


Figure 3. Titrations of anti-CD45 antibody binding to CD45+ and CD45- Jurkat cells. Specific and quantifiable binding can be seen on CD45+ cells. CD45- cells not expressing the receptor show binding levels comparable to a negative control antibody. At concentrations of antibodies present in hybridoma supernatants (1-50 µg/mL), this assay exhibits a significant signal over background.

screen is analyzed at once. Thus it is a straightforward exercise to separate the color coded cell lines, and identify antibodies that bind specifically to the CD45+ cells and those that bind in a cross reactive fashion to both CD45+ and CD45- cell lines is straightforward.

Summary

IntelliCyt's mix-and-measure hybridoma screening assay presents a powerful application to optimize the antibody screening process. Simultaneously testing antibody binding to target and control cell lines enables users to set up primary screens that combine the ability to test binding and specificity to cell surface antigens in their natural conformation. Our approach incorporates intrawell controls, adding confidence to screening results and significantly improving productivity of the hybridoma screening process. Finally, we have demonstrated here a simple implementation of color coding of cells for screening on the HTFC Screening System. Variations of this assay, including the incorporation of additional color coded cell lines in each well could be used to screen cross reactive antibodies that bind to multiple members of a receptor family or to cell surface antigens from different animal species.

References

- Garber, K. New Discoveries Still Abundant in Monoclonal Antibody Research. *JNCI J Natl Cancer Inst* (2000) 92 (18): 1462-1464.
- Chiarella P, Fazio VM., Mouse monoclonal antibodies in biological research: strategies for high-throughput production. *Biotechnol Lett.* 2008 Aug;30(8):1303-10. Epub 2008 Apr 17.

After sampling plates on the HTFC Screening System, the data were analyzed using a stepwise process with HyperView software, which facilitates a seamless transformation of raw data into screening results within minutes after data acquisition is complete. With the HyperView software, data from each plate of the

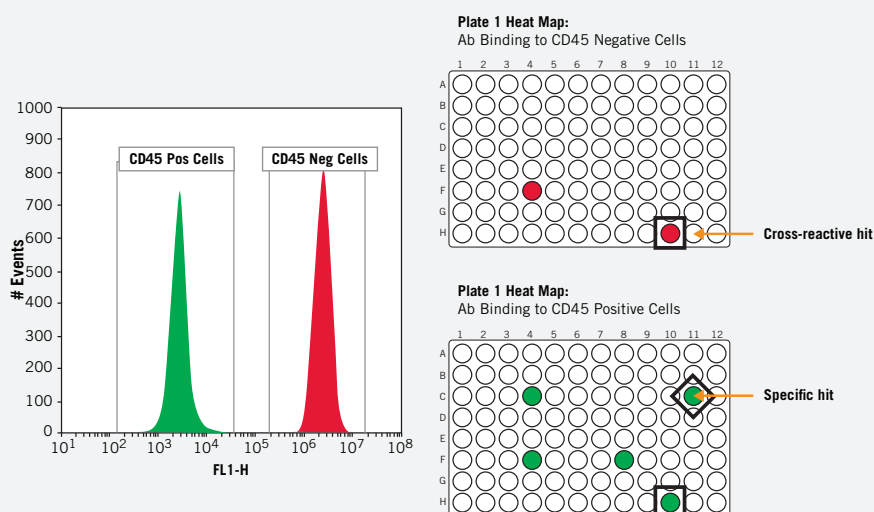


Figure 4. Target cells (unlabeled) and control cells (Calcein labeled) are mixed together, and distributed into all the wells of the assay plates. Test antibodies, such as those present in supernatants from a hybridoma library are added to the assay plates, followed by incubation with a red fluorescent detection antibody. Plates are analyzed on the HTFC Screening System, which allows the detection of binding of antibodies from the hybridoma supernatants to target and control cell populations simultaneously. The assay volume for each well was 20 µL. 1 µL of the test antibodies was added to each well.

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