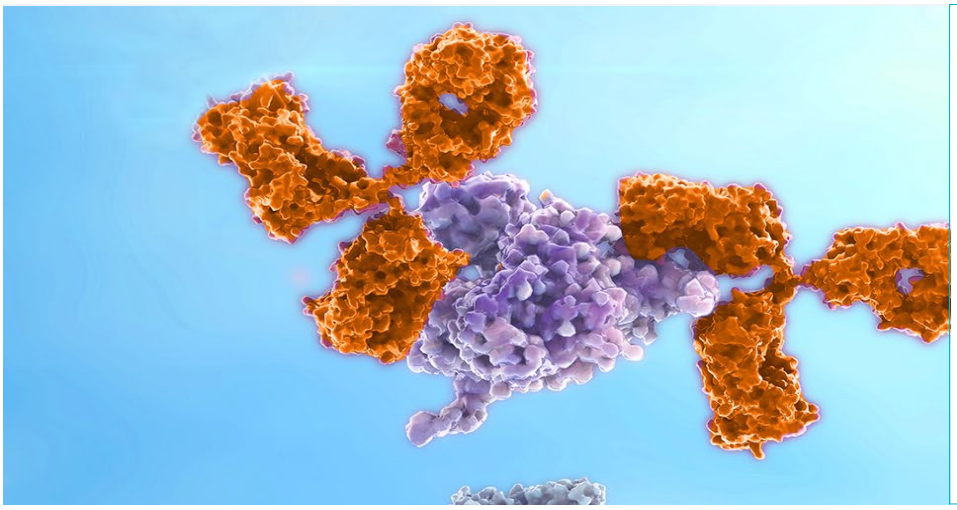
Advanced Therapeutic Antibody Discovery with Multiplexed Screening

White Paper



Scientists need powerful tools that can deliver results to fully understand the ability of candidate antibodies to interrupt cellular and molecular processes that lead to disease states.

Introduction

Antibody-based therapeutics are the fastest growing and most successful therapeutic modality for treating diseases such as cancer, cardiovascular disease, autoimmune disorders and infectious disease.[1] It is predicted that by the year 2020 there will be 70 therapeutic antibodies on the market, generating revenue of $125 billion.[2] The success of this class of drugs stems from innovative technical advances in antibody engineering and development, including display screening technologies, bispecific antibodies, and antibody drug conjugates. These technical innovations, combined with significant scientific advancements toward our understanding of the complex molecular mechanisms underlying human disease, present new opportunities for improving human health with antibody therapeutics.

The continued advancement in this field is dependent on the successful discovery and development of novel therapeutic antibody candidates. These efforts are being driven by scientists in discovery labs from pharma, biotech and academics, who need powerful tools that can deliver rapid, multifactorial results to fully understand the ability of candidate antibodies to interrupt the cellular and molecular processes leading to disease states. In this white paper, we describe how the iQue Screener, the fastest suspension based high throughput screening system available, is used to perform multiplexed screens for antibody binding to either cell surface or to circulating target antigens. We will also discuss the use of the same platform to carry out high content assays to evaluate the effects of lead candidates in multiplexed cell based and secreted protein assays.

Antibody Screening – Moving Beyond ELISA

Because therapeutic antibodies are large biologic molecules, their molecular targets are usually extracellular, either on the surface of cells or circulating in blood or other tissues. Screening hybridomas and other libraries for antibody binding to each of these types of targets has historically involved the use of enzyme-linked immunosorbent assays (ELISAs). ELISAs were developed over 40 years ago, and have been a staple in antibody screening labs. ELISAs are performed by coating a single target antigen onto the wells of assay plates, followed by addition of individual samples from an antibody library (e.g., hybridoma, phage display). Wells containing antibodies that bind to the immobilized antigen are detected through a change in color due to an indirect enzyme/substrate reaction.

While robust and relatively straightforward to run, ELISAs have a number of disadvantages that can limit the success of modern antibody screening labs:

- Primary screens that test binding to a single antigen require subsequent secondary and sometimes tertiary screens with control antigens to confirm specificity and/or crossreactivity.
- ELISAs are not ideal for screening antibodies that bind to cell surface antigens, because these antigens are extracted from the cell membrane and purified before adsorbing to the plastic ELISA plate. This often leads to disruption of conformational epitopes that can be important targets for therapeutic antibodies.
- Finally, in order to minimize background signal, ELISAs require multiple wash steps to remove unbound antibodies and detection reagents, resulting in long, laborintensive screening workflows.

"Based on flow, built for screening" philosophy combines a patented rapid microvolume plate sampling technology with a wide dynamic range, adjustment-free, detection engine. Fast, easy, high content screening.

Cell and bead-based kits for cell health and function screening. "No wash" protocol saves time; reduced volume saves money.

Full workflow support and intuitive data visualization for deep insights and better decision-making.

FIGURE 1. The iQue Screener platform features easy-to-use instruments, software, and reagent kits that are optimized to work together and designed to conserve precious samples, use less reagent, and minimize time-to-answer.

Multiplex High Throughput Screening for Antibody Discovery with iQue Screener

Recent advances in assay technology are beginning to transform the antibody discovery process. At the center of these advances is the ability to perform multiplexed, multiparameter assays at high throughput screening speeds. By collecting more information in primary screens, scientists are able to simultaneously identify hits based on specificity and cross-reactivity. Inclusion of more information early in the screening process builds confidence in potential hits, and increases the likelihood that candidate molecules will successfully proceed through downstream steps in the discovery and development process.

The iQue Screener (Figure 1) is a high throughput screening platform based on flow cytometry that rapidly processes samples in suspension. This unique capability makes the iQue Screener a powerful tool for performing high content antibody screening campaigns. It provides:

- **Content:** Multiplex assays to simultaneously test binding to target and control antigens in primary screens
- **Usability:** Antibody binding assays on either intact cell surface antigens or circulating antigens
- **Speed:** Mix and read high throughput screens

Encoding Technology – Screen Against Multiple Antigens in Every Well

The Intellicyt iQue Screener was designed to perform high throughput assays with cells and beads in suspension, which enables a powerful multiplexing approach known as encoding technology.[3] With encoding technology, scientists can combine multiple populations of cells or beads bearing different antigens of interest into the wells of assay plates, and screen against multiple target antigens in the same experiment. This greatly increases the power and robustness of antibody screening campaigns, and allows for an enhanced screening workflow. Figure 2 illustrates encoding technology and its use in antibody screening assays.

Encoding Technology for Cell- and Bead-Based Antibody Screening

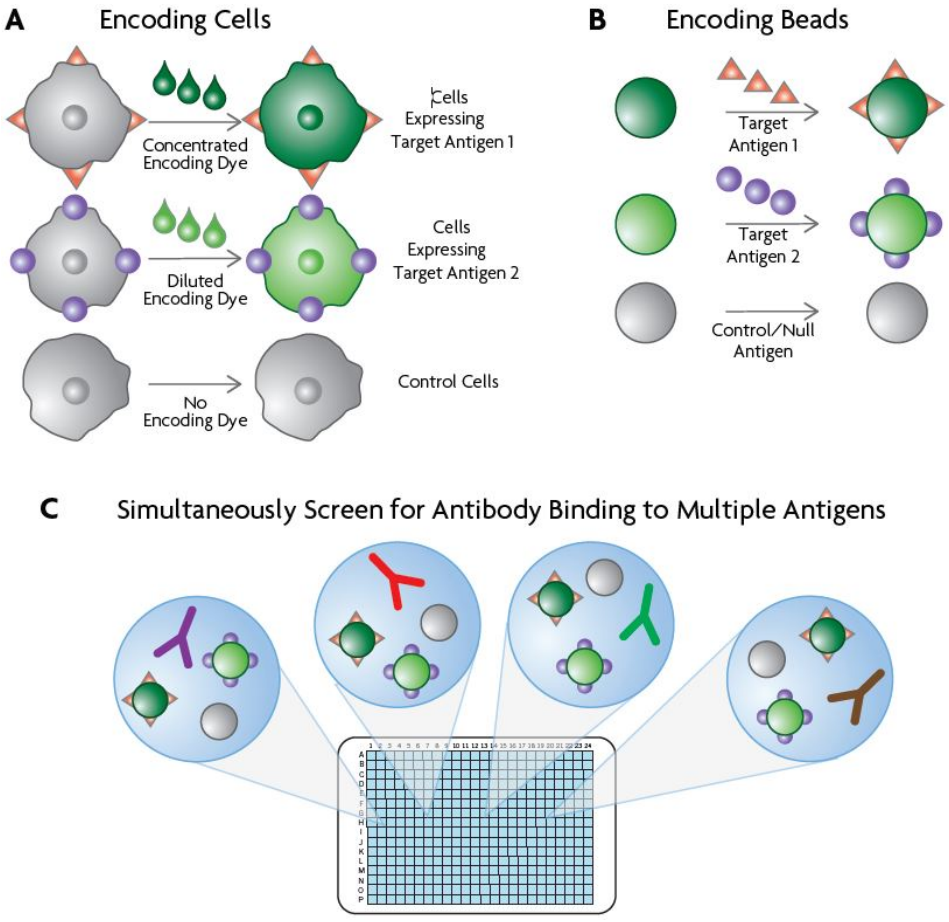


FIGURE 2. Cellular and soluble antigens can be multiplexed by using encoding technology. In the case of cellular antigens, cells expressing the various homologues are encoded with dye (A). Soluble antigens are attached to dye-encoded beads (B). The encoded cells or beads are combined and added to the screening plate (C) which contains the antibody library, and binding of the antibodies to the different antigens is measured.

Why Encode?

1. Simultaneously test antibodies for binding to target and control antigens.
This provides an internal reference control within each well, which improves confidence in hits. Also, including control antigens in primary screens can lessen the need for secondary counter screens, which streamlines the overall antibody screening workflow.

2. Screen for cross reactivity to related antigens.
Encoding allows scientists to include multiple antigens in their primary screens to search for cross-reactive antibodies that bind to families of target antigens such as cytokines. This technique can also be used to screen for antibodies that bind to a target antigen from multiple animal species. This is important when developing therapeutic candidates specific for human target antigens that can also be used in animal models of toxicity and efficacy.

3. Combine multiple projects into a single screening campaign.
Encoding can also be used to screen a single library against multiple unrelated antigens. Assay plates can be set up containing encoded cells or beads presenting different antigens of interest. Multiple antibody libraries can then be tested in the same screen, greatly improving the overall workflow

High Throughput Screens Using Encoding Technology and the iQue Screener

Following are some examples of how the iQue Screener is being used to perform more effective antibody screens, increasing the chance of discovering lead candidates that will be successful in the clinic.

Simultaneous Screening Against Target and Control Antigens on Intact Cells

In this example, two cell lines were used, one expressing a control antigen and one expressing a target antigen. Cells expressing target antigens on their surface were stained with a green fluorescent encoding dye, and cells expressing negative control antigens on their surface were left unstained. The cells were mixed together and distributed into wells of the assay plates. Test antibodies were added to the plates and following a 30 minute incubation, anti-IgG antibodies labeled with a red fluorescent dye were added to the wells to detect test antibodies bound to each cell type. After a one hour incubation, plates were read on the iQue Screener and data were analyzed using ForeCyt software (Figure 3).

ForeCyt Analysis of Multiplex, Cell Based Antibody Screen Using Encoding Technology

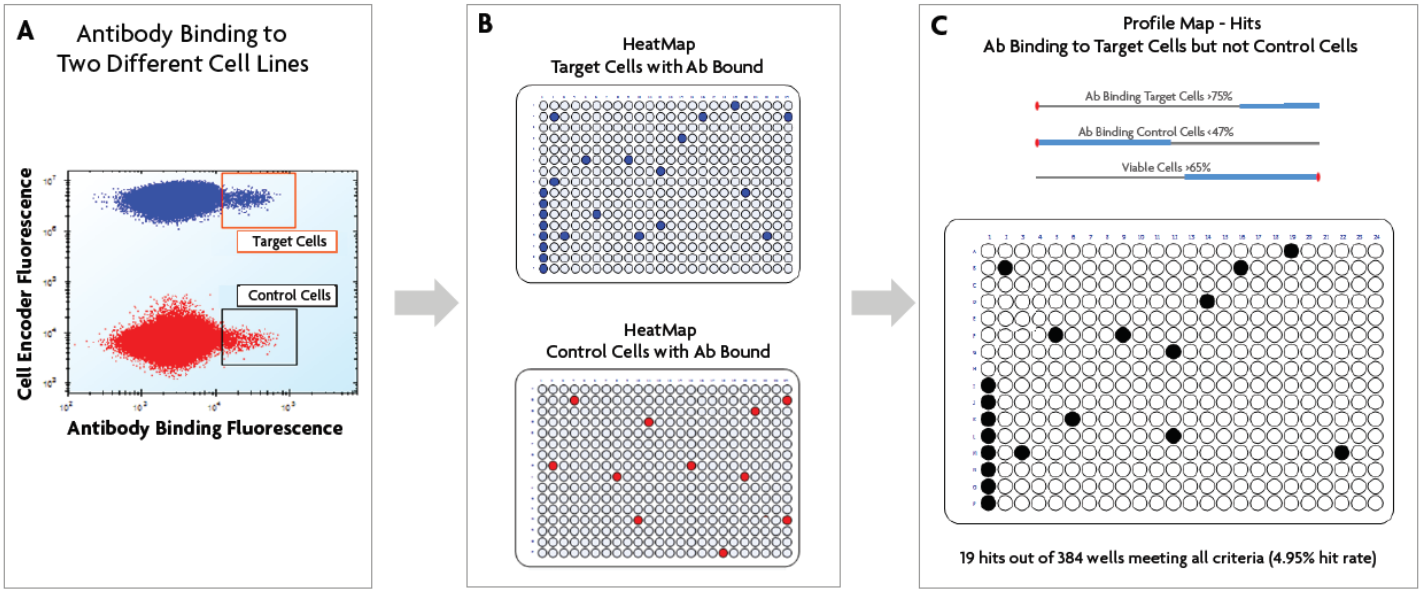


FIGURE 3. ForeCyt software adeptly analyzes data from multiplexed cell or bead based screens. Encoded cells (or beads) are discriminated in one channel (y-axis; A), and binding is discriminated in another dimension (x-axis). Heatmaps for each cell line (2 in this example) show wells in which binding occurred (B). The ProfileMap feature in ForeCyt software combines data from all selected assay parameters to show which wells meet the criteria specified for each parameter (C). In this example, wells that had binding to target cells (>75%) and control cells (<47%) as well as cell viability (>65%) are considered, and only wells meeting the 3 criteria are colored.

Screening for Cross Reactivity Using Cell Based Assays

Figure 4 demonstrates the use of multiplexed cell based assays by scientists at Xoma Corporation to identify antibodies that react with a cell surface receptor from different animal species. Plate read times of five minutes or less, combined with encoding technology, enables large scale experiments to be performed in short time frames.

Workflow for Cell-Based Species Cross-Reactivity Experiments

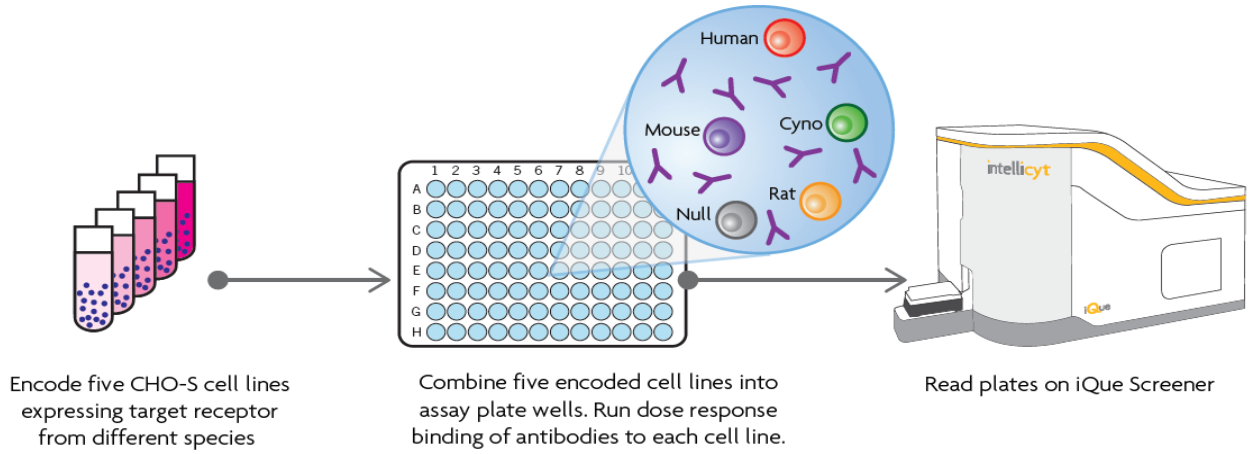


Figure 4. In these experiments, CHO cells were engineered to individually express the same target receptor from different species: human, monkey, rat and mouse (Figure 4). The parent cell line, and lines expressing the species-specific receptors were labeled separately with different concentrations of the encoding dye. The five different cell lines were combined into a single mixture, which was then distributed into wells of the assay plates. After sampling, the differentially encoded cell lines within each well were easily distinguished using ForeCyt software, which enabled the identification of crossreactive antibodies. In these experiments, each 96-well assay plate contained 12 antibodies, tested at 8 concentrations each. Each well contained five encoded cell lines.

Representative Data From Cross-Reactivity Experiments for 12 Antibodies

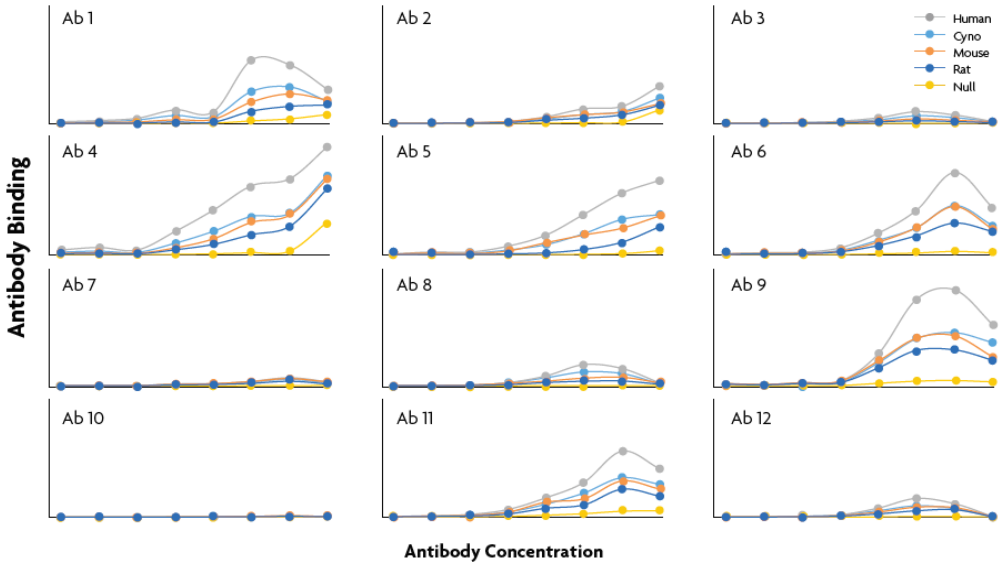


Figure 5. Cross-reactivity experiments enable the rapid discovery of antibodies that bind to both human and animal receptor homologues. Using this approach, potential drug candidates that can be used in humans and also be tested in preclinical models of efficacy and toxicity.

Screening for Cross Reactivity Using Bead Based Assays

Affimers developed by Avacta Life Sciences are small engineered binding molecules designed to be more robust than antibodies, and with Intellicyt technology they can be generated very rapidly. Figure 6 demonstrates the use of multiplex bead-based assays by scientists at Avacta to screen affimer libraries generated from phage display panning outputs for cross-reactive binders to different mouse and human immune checkpoint molecules. Compared to running traditional ELISA based format, combining 5 binding assays in each well reduced the amount of protein required for each target 166-fold, the number of plates required for the total screen 20-fold and reduced the total primary screen time 5-fold. The time to perform the entire screen was less than one hour. Running this same screen using single-plex ELISA assays would have required ten 384-well plates (or forty 96 well plates) and several mg of each target protein.

Avacta's Affimer Screening Workflow

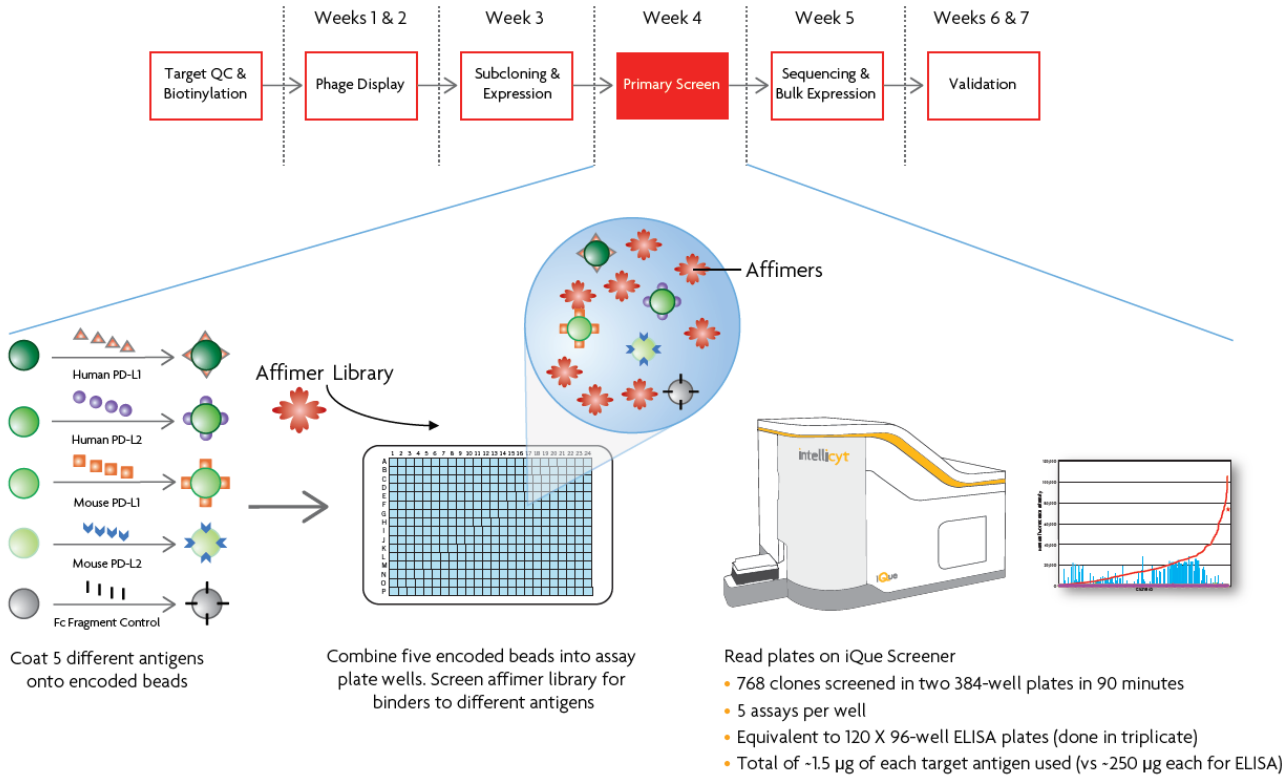


Figure 6. Encoded beads were coated with four different checkpoint modulating proteins: human PD-L1, human PD-L2, mouse PD-L1, and mouse PD-L2. Since these proteins were expressed as Fc fusions, a fifth encoded bead was coated with Fc fragment, and served as a negative control (Figure 6). The five beads were combined into a single mixture, which was then distributed into wells of 384-well assay plates. A library consisting of 768 affimers selected by panning against human PD-L1 was screened for binding to the different PD-L1 and PD-L2 antigens.

Using multiplex bead based assays, Avacta is employing large scale experiments on the iQue Screener platform to significantly improve their affimer screening workflow (Figure 7).

Multiplex Screening Data Identifying Cross-Reactive Affimers for PD-L1

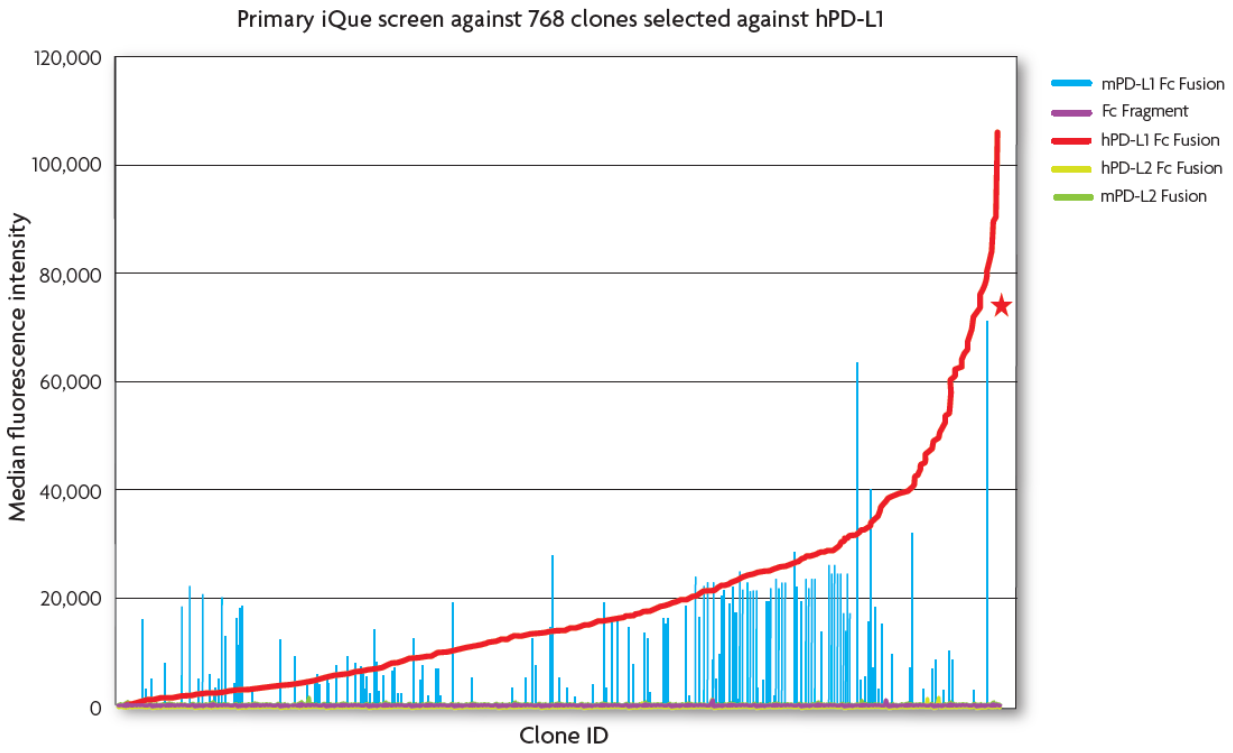


Figure 7. The screen data shows the identification of clones that bound to both human PD-L1 (red line) and mouse PD-L1 (light blue bars), but not to human or mouse PD-L2 (green and grey bars, respectively). No clones that bound to control beads coated with the Fc fragment were identified (purple bars). The star indicates one clone with high affinity for both human and mouse PD-L1.

Benefits of the iQue Screener Platform

Encoding technology enables the development of robust multiplex screening assays to improve chances of identifying quality antibody candidates and improve screening workflows.

- Greatly reduce antigen usage
- Include references and controls in every well
- Screen for cross reactivity
- Screen for cross reactivity
- Combine multiple libraries in a single screen

The iQue Screener is a flexible system for performing high throughput, multiplexed screens for circulating or cell surface antigens.

- Screens for antibodies against circulating antigens such as cytokines cytokines and other biomarkers can be performed with target antigens coated on multiple encoded beads.
- Cell based assays using encoded cells can be used to screen for antibody binding to multiple surface antigens presented in their natural conformation in intact cells.

Additional benefits of multiplexed antibody screening with the iQue Screener:

- Rapid plate read times: 96-well plates < 5 minutes, 384-well plates < 20 minutes.
- Binding assays can be performed with no-wash, mix and read protocols.
- ForeCyt software on the iQue Screener is a powerful, easy to use analysis tool that enables rapid identification of quality hits.

Summary

Multiplexed high throughput screening has become an essential component in the discovery of therapeutic antibodies. Scientists are using the iQue Screener platform to perform powerful high content screens for their antibody libraries. The iQue Screener platform enables robust encoding technology, saves valuable reagents, has powerful analytical software for lead identification and provides the fastest time to answer for antibody discovery.

References

1. Chames, P., et al. (2009) "Therapeutic antibodies: successes, limitations, and hopes for the future." *British Journal of Pharmacology* 157: 220-233.
2. Ecker, D.M., et al. (2015) "The therapeutic monoclonal antibody market." mAbs 7: 9-14.
3. Black, C.B., et al. (2011) "Cell-Based Screening Using High-Throughput Flow Cytometry." *Assay and Drug Development Technologies* 9:13-20.

Intellicyt Coporation,
a Sartorius Company
5700 Pasadena Ave
Albuquerque, New Mexico 87121
USA

www.intellicyt.com

USA +1.505.345.9075

Email: info.intellicyt@sartorius.com

FOR RESEARCH USE ONLY. NOT for IN VITRO DIAGNOSTIC USE

Intellicyt technology is protected by the following patents and other patents pending:6,890,487, 6,878,556, 7,368,084, 7,842,244, 8,021,872, 8,268,571, 8,637,261, 8,823,943, 9,012,235, D,722,515

© 2018 Intellicyt Corporation. All rights reserved. iQue, Intellicyt Corporation and all names of Intellicyt Corporation products are registered trademarks and the property of Intellicyt Corporation unless otherwise specified. Intellicyt Corporation is a Sartorius Company.
Document No.: 11943.D