intellicyt®

# Better Antibody Screening

## Reduce costs. Reduce false negatives. Improve success.

### Introduction

With the increasing need for new therapies for cancer, autoimmune disorders and Alzheimer's disease, monoclonal antibodies are proving very successful due to their high affinity and specificity for their target antigens. In cancer, for example, their first use was as antagonists of oncogenic receptor tyrosine kinases, but today monoclonal antibodies have emerged as vehicles for the targeted delivery of potent chemotherapeutic agents and as powerful tools to manipulate anticancer immune responses.[1] Currently, more than 30 antibody therapeutics are marketed worldwide. Some analysts predict that, given the rate of current revenue growth and potential new approvals, the global market could reach $58 billion by 2016.[2] In addition, there are hundreds of monoclonal antibody-based biologic drugs in various phases of clinical trials worldwide.

The success of biologic drugs such as rituximab (Rituxan), cetiximab (Erbitux), bevacizumab (Avastin), ibritumomab (Zevalin) has driven the race to discover new monoclonal antibodies at pharmaceutical, biopharmaceutical and academic labs worldwide. However, screening hybridoma libraries is a critical bottleneck in biologics discovery. It is both a time and resource intensive process that often involves several sequential testing steps using multiple technologies. In this white paper, we discuss numerous challenges contributing to this bottleneck, and provide solutions to overcome these issues that ultimately enhance the antibody screening processes.


**Figure 1. Traditional Antibody Screening Process.**

### The Traditional Antibody Screening Process

The antibody screening process for cell surface target antigens traditionally consists of a series of steps to sequentially determine binding and specificity using biochemical assays, followed by further binding assays using cell-based assays (Figure 1). The first binding screen is performed with an ELISA where the target antigen is purified, isolated, and then immobilized on the surface of an assay plate. Once binders are found in this primary screen, subsequent specificity testing is required to ensure that the antibodies do not cross-react with other antigens. Positive antibodies are then advanced and further tested using cell-based assays to determine if they bind to the antigen in the more physiologically relevant environment of the cell membrane.

### The Challenges of ELISA

ELISA has been the backbone of the antibody screening process for many years, and provides a reliable and convenient method for measuring antibody-antigen interactions. It has been the gold standard in protein analysis. However, some of the most important classes of drug targets are cell surface and membrane associated proteins, including G-protein coupled receptors (GPCRs).

Using an ELISA method for cell surface antigens poses multiple challenges. These include:

- Purifiying the antigens in a conformationally correct format
- Immobilization of the antigen protein to the surface of the ELISA plate

Utilizing purified cell surface proteins in ELISA assays can result in significant changes to the conformation and functionality of the antigen. An approach that measures binding of candidate antibodies in intact, functional cells would be a major advantage for the biologics discovery process.

## General Workflow Issues

Looking at the traditional antibody screening process, we can organize the steps into a series of sequential assays as follows:

- **Primary Screens**
  - *Assessments of binding and specificity with biochemical assays*
- **Secondary Screens**
  - *Cell-based binding assays*
- **Functional Assays**
  - *Confirming if the antibody binding to its target results in the desired biological effect*
- **Optimization Screens**
  - *Affinity maturation and humanization which includes three steps: an optimization binding assay, a specificity assay and a final cell-based binding assay*

This overall workflow is resource-intensive in terms of time, the requirement for multiple instrument platforms, and the costs associated with sourcing reagents from multiple vendors. The antibody screening process typically involves biochemical assays in the primary screen. This approach, which uses denatured proteins, can lead to potential missed hits. Furthermore, each of the various steps in this sequential testing process contributes to variability in results and introduces the potential for error. Workflows that could provide increased data consistency, reduce costs, and shorten time requirements would help streamline the search for new monoclonal biologic drugs.

## Characteristics of a Better Antibody Screening Process

ELISA and Fluorometric Microvolume Assay Technology (FMAT)[3] have been widely used in antibody screening. However, advances that improve the process by addressing the limitations we have presented are in demand by the screening community. An ideal antibody screening process would include the following features:

- Allow for the use of intact cells in order to present cell surface antigen targets in their natural conformation
- Reduce both the number of steps and overall workflow complexity for the hybridoma screening process
- Incorporate multiplexing in order to measure multiple targets and species in the same assay at the same time

## Using IntelliCyt's High Throughput Flow for Improved Antibody Screening

In the past, flow cytometry was considered a viable approach to hybridoma screening[4], but multiple assay steps and low throughput were regarded as a disadvantage. With IntelliCyt's high throughput flow technology, cell-based assays can be used throughout the screening process, eliminating biochemical assays and improving results.

**The IntelliCyt Approach:**

- Offers high throughput screening capabilities, measuring a 96 well plate in as little as three minutes
- Assays proteins in their natural conformation by using intact cells
- Employs multiplexing which simultaneously assesses binding, specificity and cross-reactivity of multiple cell lines in the same well
- Provides a simple no-wash format that reduces workflow steps and costs compared to standard ELISA

The IntelliCyt approach uses **intact cells** with the antigen in its **natural conformation**, significantly reducing the risk that a promising potential hit will be missed (Figure 2).

Importantly, the IntelliCyt approach allows **simultaneous testing of antibody binding to target and control cell lines** in the same well. This enables design of primary screens that combine the ability to test both binding and specificity to cell surface antigens, and do so in fewer steps compared to traditional ELISA. By color coding target cells and negative control cells, antibodies binding specifically to the target cells and not to the control cells are quickly and

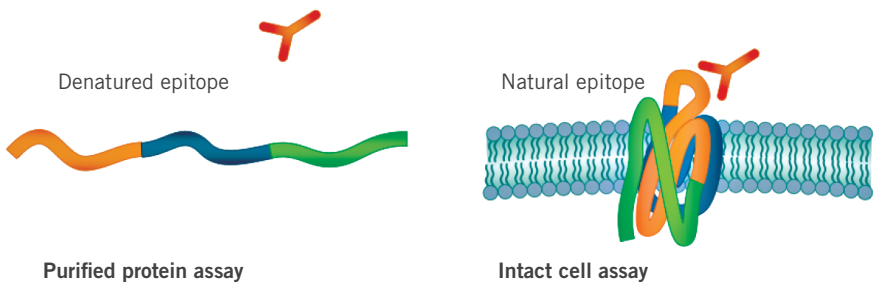



**Figure 2. Intact cell-based assays allow screening of antibodies to conformational epitopes that may not be available in ELISAs and other purified protein assays where the antigens are often unfolded and denatured during the purification process.**

accurately determined. In the example shown in Figure 3, target cells (unlabeled) and control cells (green fluorescent labeled) were mixed together and distributed into all the wells of a set of assay plates. Test antibodies, such as those present in supernatants from a hybridoma library, were added to the assay plates followed by incubation with a red fluorescent detection antibody. Cell-by-cell detection using high throughput flow enables precise analysis of hybridoma binding to target and control cells. The protocol requires only two incubations, reducing the three steps typically required by ELISA down to only one step - providing a much simpler and less error-prone workflow.

### A new tool for antibody screening

IntelliCyt Corporation is the first to commercialize a system that enables exclusively using cells for both the primary and optimization screen. IntelliCyt's screening systems, including its flagship iQue® Screener, use high throughput flow technology to rapidly assay cells in suspension using flow cytometry as the readout. The technology also has broad applications in drug discovery screening, enabling high throughput, high content evaluation of individual cells and beads.

## IMPROVED ANTIBODY DISCOVERY IN ACTION

*A growing number of biotechnology companies have experienced demonstrated advantages for better antibody discovery by adopting IntelliCyt's technology.*

### Developing Highly Specific Antibodies to GPCRs

**Morphosys**, a world leading biotechnology company with a rich pipeline of future therapeutic antibody products, has generated antibodies against GPCRs in their native conformation using the IntelliCyt technology. A typical screening campaign for Morphosys comprises greater than 10,000 clones. They needed a system that could screen multiple parameters simultaneously and quickly in order to help eliminate antibodies binding to irrelevant cell surface proteins. The iQue Screener provided the rich data they needed to increase their chances of finding GPCRs that were optimal therapeutic candidates.[5]

### Generating Monoclonal Antibodies to Transmembrane Proteins

**SDIX**, an OriGene Company, has developed technologies comprising an antibody engine (mAb discovery engine) for discovery of antibodies to transmembrane proteins like GPCRs. The mAb discovery engine generates panels of diverse antibodies in terms of sequence, somatic hypermutation, epitopes, and performance. The platform includes DNA immunization technology (vectors, response modifiers, protocols), IntelliCyt's high throughput flow technology, cell constructs, screening assays including functional analyses, antibody gene sequencing and expression, and epitope mapping. The mAb discovery engine has enabled SDIX to generate first-in-class and best-in-class antibodies against important and challenging therapeutic targets[6].

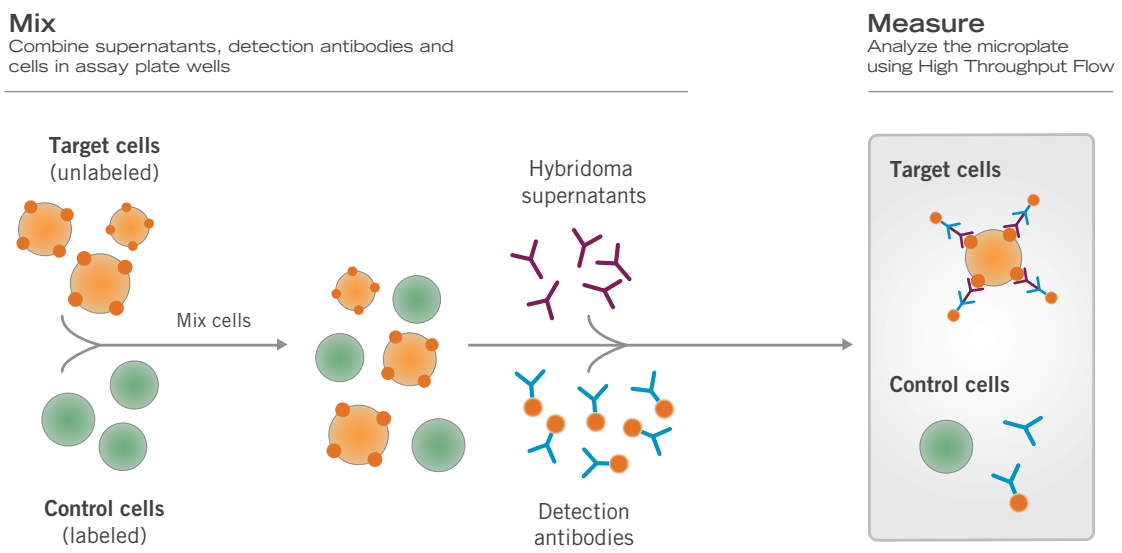



**Figure 3. Mix and measure format reduces workflow steps.**

## Systems designed for high throughput, multiplexed screening

IntelliCyt screening systems send a continuous sample stream to the laser-based fluorescence detector, which collects multiple readouts from individual cells. The system employs a unique sampling method to transfer cells or other materials from microplate wells to the detector in a continuous air gap-delimited stream. This novel method confers the following advantages:

- Assays a 96 well plate in as little as 3 minutes; 384 and 1536 well operation are also available
- Samples ~10,000 objects / second
- Uses sampled volumes as low as 1 µL; assay volume as low as 10 µL
- Eliminates dead volume usually associated with flow cytometry
- Achieves high sensitivity across a dynamic range of 6+ decades
- Minimizes cross-contamination

## Summary

Adopting high throughput flow technology enables significant improvements in the antibody screening process.

Specificity and affinity assays traditionally performed separately can be combined using intact cells, including mixtures of different cell types (target and control cells or cells from multiple species). With intact cells, epitopes can be presented more realistically, improving results. IntelliCyt's systems allow high throughput, low volume screening of these cellular assays, providing a cost-effective and technically superior alternative to ELISA and FMAT assays.

The IntelliCyt technology has shown demonstrated success in biopharmaceutical companies to improve screening from phage libraries and hybridomas, especially for antibodies against transmembrane proteins.

For more information visit **www.intellicyt.com**

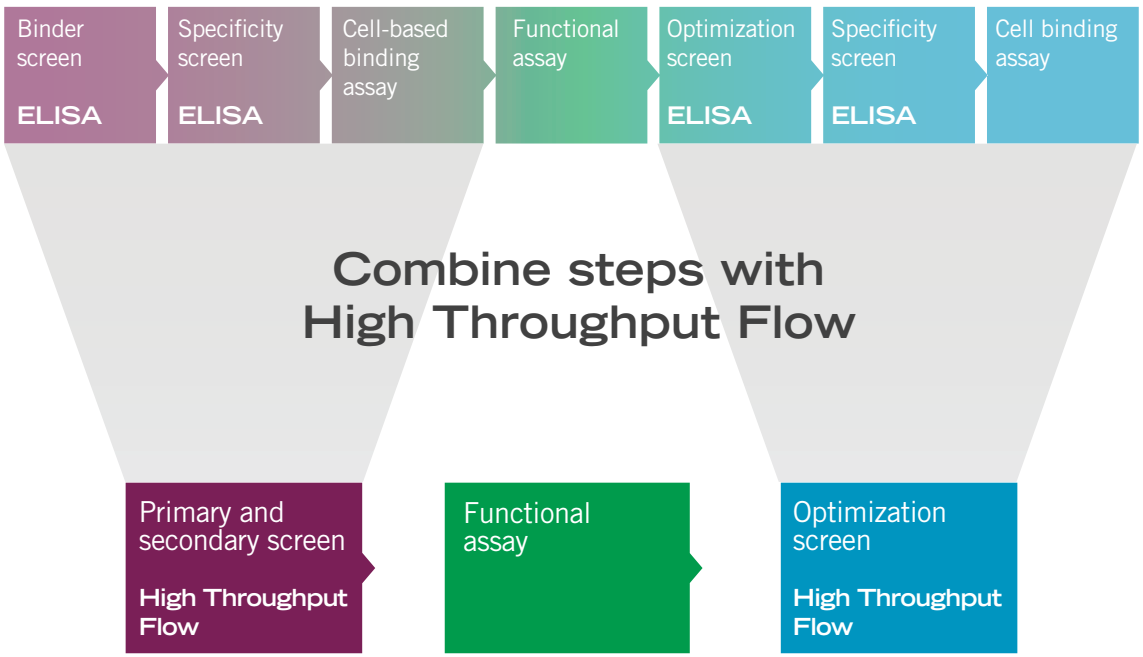


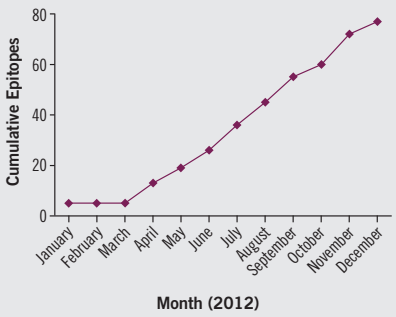

**Figure 4. Significantly improve antibody discovery at two critical points in the development process with IntelliCyt's high throughput flow technology.**

## IMPROVED ANTIBODY DISCOVERY IN ACTION

*A growing number of biotechnology companies have experienced demonstrated advantages for better antibody discovery by adopting IntelliCyt's technology.*

### Epitope Mapping and Protein Engineering Programs for Membrane Protein Targets

**Integral Molecular**, a provider of innovative solutions for scientific research and drug discovery applications involving cellular and viral integral membrane proteins, has employed the IntelliCyt technology for their epitope mapping programs. They have developed a technology called Shotgun Mutagenesis, a novel strategy for epitope mapping that rapidly evaluates functional effects of point mutations across an entire target protein. The iQue Screener helped them evaluate thousands of point mutations to a target protein for antibody binding, enabling identification of amino acids critical for the epitope with a savings in both time and reagent costs. The lab experienced an increase in both capacity and productivity. The antibody screen is more effective and specific, since only epitopes available within the context of the cell surface are exposed. This enables the expression of mutants in live human cells while maintaining native structure and modifications. In addition to epitope mapping, their shotgun mutagenesis tool has been utilized in both antibody and protein engineering.[7]




**Increase in productivity for Integral Molecular after adopting IntelliCyt's high throughput flow technology in March 2012.**

### Incorporating Multiple Species Cross Reactivity for Lead Drug Candidates

**XOMA, LLC** uses proprietary technology to discover, develop and manufacture novel antibody therapeutics in areas including cardiometabolic, inflammatory, autoimmune, infectious disease and cancer. Antibody therapeutics are unique in that they are exquisitely specific in terms of their binding to the antigen. This presents challenges downstream in the drug discovery process when a lead candidate is selected for human use, but it may have limited binding in animal species. This makes pre-clinical testing difficult. To overcome this obstacle, Xoma has used the power of multiplexing in the IntelliCyt technology to incorporate cross species reactivity of antibodies early into the screening phase of the discovery process. This process helps ensure less variability and aids in the selection of viable therapeutic candidates.[8]

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