High-Throughput Screening for Antibody Discovery Using Mirrorball

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Antibody Engineering & Therapeutics
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Christyne Kane is an employee of AbbVie.

Assessment of equipment is the opinion of the author.

AbbVie does not endorse any of the platforms described in this presentation.
Overview

Antibody Discovery: Mirrorball HTS

Antibody Screening Assays

- Isotype Determination: Bead Color Discrimination
- Cell Surface Binding
  - Singleplex
  - Multiplex
- Cell Killing Assay
- ELISA Alternative: Bead Size Discrimination
High-Throughput Screening for Antibody Discovery

**Desired Antibody Properties**

- Soluble Target Binding
- Cell Surface Antigen Binding
- Species Cross-reactivity
- Target Specificity
- Functionality
- Epitope Binning
- Isotype

**Use of Mirrorball Technology**
Mirrorball Technology

Mirrorball Instrument Configuration

3 LED lasers
- 405nm
- 488nm
- 640nm

4 Fluorescent Channels
- FL-1 (420-488nm)
- FL-2 (488-540nm)
- FL-3 (560-610nm)
- FL-4 (650-690nm)

1 Scatter Channel (SC-1)

Automation: Thermo Scientific Orbiter RS

Benefits of Mirrorball

- **HOMOGENEOUS ASSAY** - No wash steps
- Ability to **MULTIPLEX** for multiple antigens, cross-reactivity, or counter-screening
- Compatible with **CONDITIONED MEDIA**
- **SENSITIVITY** (adequate for low concentration supernatants)
- +/- **HIT ASSESSMENT**, flexible data analysis
- Integrates with **AUTOMATION**, miniaturization for HTS
- **TIME SENSITIVE** (24-hour turnaround)
- **PLUG-AND-PLAY** assay for multiple targets
Case Study: Target A

Target Biology

- TNF family member
- Transmembrane glycoprotein
- Forms a homotrimer
- Autoimmune indications

Goal: To generate a panel of antibodies binding cell-surface Target A.

Desired Binding Properties

- Binding to specific epitope
- Binding to cell surface protein

Immunization Strategy

- Peptide immunizations in mice
Multiplexed Screening Enabled by Bead Size Discrimination

Mix-and-Read Assay Setup

Compare to ELISA

- 384 well plate format
- No washing steps
- Shorter incubation times
- Smaller volumes of reagents
- Multiplex-able

Beads are Distinguishable

Specific Binding Detectable

Compare to ELISA

- 384 well plate format
- No washing steps
- Shorter incubation times
- Smaller volumes of reagents
- Multiplex-able

Bead Size

High-Throughput Screening for Antibody Discovery using Mirrorball | Date 12.12.17 | © 2017
Hybridoma HTS by Mirrorball Completely Correlates with ELISA

Mirrorball vs. ELISA

- Hit by Mirrorball & ELISA
- Hit by neither

100% Correlation
- Singleplex assay using 7μm beads
- No false positives
- No false negatives
Screening Methods are in Agreement for Hit Identification

<table>
<thead>
<tr>
<th>Screening Assay</th>
<th>Number of mAbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Screening (ELISA)</td>
<td>153</td>
</tr>
<tr>
<td>ELISA</td>
<td>38</td>
</tr>
<tr>
<td>Flow</td>
<td>38</td>
</tr>
<tr>
<td>Mirrorball</td>
<td>38</td>
</tr>
</tbody>
</table>

**Summary**

- Bead-based binding assay correlated with ELISA methods 100% for early supernatant screening.
- 8 antibodies identified bound specifically to Target A by flow cytometry.
Case Study: Target B

**Target Biology**

- Soluble protein
- Neurology and cardiovascular indications

**Goal:** To generate a panel of antibodies binding Target B.

**Desired Binding Properties**

- Binding to target B protein
- +/- Binding to target protein family members

**Immunization Strategy**

- Protein immunizations in mice
Direct Isotype Determination Enabled by Sixplex Bead-based Assay

Mix-and-Read Assay Setup

**AF488**
- Anti-Species
- Light Chain

**Test Antibody**
- Anti-Species HC
- SA/Biotin

**SA/Biotin**

**IgG2b**
- sol-R2

**IgG3**
- sol-R3

**IgG2a**
- sol-R4

**IgG1**
- sol-R5

**AF405/BV421**
- *LC-specificity*
- (optional)

Beads are Distinguishable

Specific Binding Detectable

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# HC & LC Isotypes are Clearly Detected in Hybridoma Supernatant

<table>
<thead>
<tr>
<th>Antibody</th>
<th>HC Isotype</th>
<th>LC Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb 1</td>
<td>IgG3</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 2</td>
<td>IgG1</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 3</td>
<td>IgG2a</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 4</td>
<td>IgG2a</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 5</td>
<td>IgG1</td>
<td>Lambda</td>
</tr>
<tr>
<td>mAb 6</td>
<td>IgG2a</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 7</td>
<td>IgG2a</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 8</td>
<td>IgG2a</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 9</td>
<td>IgG2a</td>
<td>Kappa</td>
</tr>
<tr>
<td>mAb 10</td>
<td>IgG3</td>
<td>Kappa</td>
</tr>
</tbody>
</table>

**Able to detect multiple isotypes per sample**

---

Kappa

Lambda

---

Able to detect multiple isotypes per sample
HT Isotype Determination Improves Upon Traditional ELISA

**ELISA Isotyping**

<table>
<thead>
<tr>
<th>Isotype</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG2a</td>
<td>mAb 1</td>
</tr>
<tr>
<td>IgG2a</td>
<td>mAb 2</td>
</tr>
<tr>
<td>IgG2a</td>
<td>mAb 3</td>
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<td>IgG2a</td>
<td>mAb 9</td>
</tr>
<tr>
<td>IgG3</td>
<td>mAb 10</td>
</tr>
</tbody>
</table>

**Mirrorball Isotyping**

Non-specific signals have potential to dilute or overpower specific signals, leading to incorrect isotype conclusions.

Specific signals are greater than all background levels, allowing for accurate determination of isotype results.
Case Study: Target C

Target Biology
- 7-Transmembrane GPCR with short extracellular loops.
- Small molecule antagonists and agonists are available.
- Unprecedented for targeting by biologics

Goal: To generate a panel of antibodies binding cell surface Target C which can be internalized.

Desired Binding Properties
- Binding to cell surface
- Cross reactivity to cyno
- Cross reactivity to rat

Desired Biological Activity
- Cell Killing

Immunization Strategy
- cDNA immunizations in rats
Singleplex Cell Binding Assay Enables HT Supernatant Screening

Mix-and-Read Assay Setup

GFP+ Cells are Detectable

Target-Specific Ab

Target-Expressing Cell Line (GFP+)

AF647 Anti-Species

Target Antigen

AF647

GFP

Merge

Specific Binding Detectable

(-) Binding

(+ ) Binding

GFP Mean Intensity (FL1-H)

PL-2 Mean Intensity (FL1-H)
Target C Campaign Enabled by Mirrorball Antibody Screening

<table>
<thead>
<tr>
<th>Mirrorball</th>
<th>Number of mAbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target C Species</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Cyno</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
</tr>
<tr>
<td>Cell Killing</td>
<td>22/28 tested</td>
</tr>
</tbody>
</table>

Flow Cytometry Binding to Target C

Summary

- Single-plex cell binding assay enabled all stages of supernatant screening.
- Antibodies identified bound specifically to Target C by flow cytometry and had a range of functional potencies.
Case Study: Target D

Target Biology

- TNF family member
- Transmembrane glycoprotein
- Forms a homotrimer

Desired Binding Properties

- Binding to cell-surface protein
- Cross-reactivity to Cyno

Desired Biological Activity

- Block ligand/receptor interactions

Immunization Strategy

- Whole cell immunizations in mice

Goal: To generate a panel of antibodies binding Target D which blocks interaction with receptor.
Multiplexed Cell Binding Assay Enables HT Supernatant Screening

**Mix-and-Read Assay Setup**

- 384 well plate format
- No washing steps
- Shorter incubation times
- Smaller volumes of reagents
- Fewer cells required
- Multiplex-able

**Cell Types are Distinguishable**

**Compare to Flow Cytometry**

- 384 well plate format
- No washing steps
- Shorter incubation times
- Smaller volumes of reagents
- Fewer cells required
- Multiplex-able

**Specific Binding Detectable**

**Transfected**

**Parental**

**Parental Cell Line**

**Target-Expressing Cell Line**

100nM Far Red

1000nM Far Red

**AF488 Anti-Species**

**Target-Specific Ab**

**Target Antigen**

**AF488**

**Far Red**
Hybridoma HTS by Mirrorball: 89% Correlation with Flow Cytometry

Mirrorball vs. Flow Cytometry

- Hit by Mirrorball & Flow Cytometry
- Hit by Flow Cytometry only
- Hit by neither

89% Correlation

- Mirrorball missed 5/45 hits by Flow Cytometry (false negatives)
- No false positives
Multiplexed Cell Binding Assay is Implemented for Sub-clone Screening

Multiplexed cell binding assay demonstrated an 89% correlation with Flow cytometry methods.

All downstream sub-clone screening was performed using the Mirrorball multiplexed cell binding assay, enabling the delivery of 8 antibodies.

Summary

- Multiplexed cell binding assay demonstrated an 89% correlation with Flow cytometry methods.
- All downstream sub-clone screening was performed using the Mirrorball multiplexed cell binding assay, enabling the delivery of 8 antibodies.
Case Study: Target E

Target Biology

- GPI-linked, glycosylated, membrane protein.
- Modulates T cell and macrophage activation.
- Oncology indications

Goal: To generate a panel of antibodies binding cell surface Target E which can be internalized.

Desired Binding Properties

- Binding to cell surface target E
- Cross-reactivity to Cyno
- Cross-reactivity to Mouse

Desired Biological Activity

- Internalization

Immunization Strategy

- cDNA immunizations in mice
High Throughput Cell Killing Assay

Mix-and-Read Assay Setup

Detection | Live | Apoptotic* | Dead
---|---|---|---
Hoechst | ✓ | ✓ | ✓
Annexin V-AF488* | ✓ | | |
TO-PRO-3 | | ✓ | |

*Optional

Well View

Cells & Media
Cells & Ab Complex

SMi
Anti-Species Ab
Target-Specific Ab
Target Antigen
Target-Expressing Cell Line

~72-96hrs
Target-specific Cell Killing is Detectable

**Plate View**

**Example Data**

% Viability =

\[
100 - \frac{\text{# Dead Cells} + \text{# Apoptotic Cells}}{\text{# Total Cells}}
\]

Summary

- Target-specific killing is detectable.
- No non-specific killing is observed.
Cell Killing Assay Enables HT Detection of Internalizing Antibodies

### Screening of Hybridoma Supernatants

![Graph showing cell viability across different concentrations of hybridoma supernatants.](image)

### Platform Advantages

- Compatible with early hybridoma screening.
- Automated for high-throughput assay set-up and data reporting.
- Streamlined process to identify functional leads.
Antibody Screening Assays: Saving Time, Labor & Reagents

ELISA Alternative: Bead Size Discrimination
• Plug-and-play assay is easily established, requiring smaller amounts of reagents and significantly less time, compared to traditional ELISA.

Isotype Determination: Bead Color Discrimination
• Color-coded sol-R beads enable the establishment of a robust multiplex assay able to identify 4-8 unique antibody properties with increased sensitivity compared to traditional methods.
• Format may be applied to cross-reactivity and specificity assays.

Cell Surface Binding
• No wash assays require 1/10th the cells of traditional flow cytometry screening and may be automated at all steps, including data processing.

Cell Killing
• High throughput cell killing assay allows for functional screening of hybridoma supernatants using a small amount of material and a variety of secondary-conjugated toxins.

Antibody Discovery: Mirrorball HTS
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