Automated and High-throughput Antibody Production, Validation and Characterization at the Institute for Protein Innovation (IPI)

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Institute for Protein Innovation

Introduction

Monoclonal antibodies play critical roles in diagnosis, treatment and biomedical research. While therapeutic antibodies are stringently validated, reagent antibodies are typically not tested to the same degree. The lack of validation and information can lead to potential issues with performance and reproducibility. At the Institute for Protein Innovation (IPI), we are dedicated to addressing this issue by developing high-quality and well-characterized synthetic recombinant antibody reagents that drive advancements in biomedical research.

To achieve this, we utilize yeast display technology to identify antibody candidates with variations in the heavy chain CDR3 region, clustering them via next-generation sequencing. This highly efficient Antibody Discovery Platform routinely generates hundreds of hIgG1 antibodies. To identify the best-performing antibodies, we leverage state-of-the-art automation and high-throughput methodologies with multiple robotic liquid-handling workstations to produce, validate and characterize large sets of antibodies. Our antibodies were produced through automated 96-well transient transfections followed by high-throughput purifications. For validations, we devised ELISA, flow cytometry, and bio-layer interferometry (BLI) assays in 384-well formats, using robotic workstations to perform titration, plate-rearrangement, plate wash, and reagent application, ensuring the rapid analysis in productivity, non-specificity, stability, kinetics and affinity.

The integration of automated systems allows us to carry out various tasks with efficiency, consistency, precision and reproducibility. These high-throughput technologies allow us to process large numbers of antibodies rapidly and simultaneously, enabling us to deliver reliable antibody products to demanding targets.

Antibody Production

Transfection

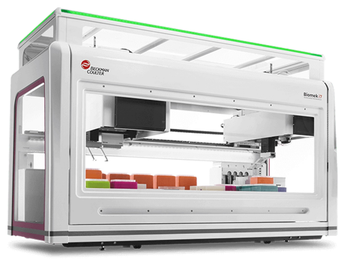
- **Daily throughput:**
  Two 96-well plates in duplicates

Productivity Assessment

- **Daily throughput:**
  Two 96-well screening plates
- **Assay (ValitaTITER):**
  Florescence polarization-based IgG quantitation in cell culture
- **Purpose:**
  To quickly estimate the success of the production process

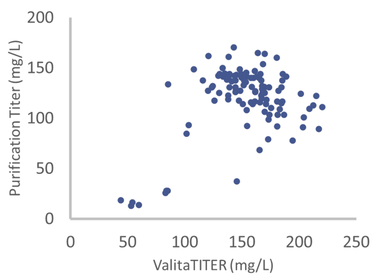
Purification

- **Daily throughput:**
  Two 96-well screening plates
- **Purification strategy:**
  Magnetic Protein A resins



Biomek i7 (*Beckman Coulter*)

An automated workstation widely used at IPI for 96-well plate transfections, 96-well plate purifications and 50 mL bioreactor transfections.



Productivity Result

Majority of the antibody candidates have adequate production yields

Antibody Quality Control

Poly-Specific Reactivity (PSR)

- **Daily throughput:**
  One 384-well ELISA plate
- **Assay (PSR-ELISA):**
  ELISA-based single-point affinity evaluation against poly-specific reagents
- **Purpose:**
  To assess the non-specificity of antibody candidates
- **Automated workstation:**
  Biomek NX^{P} (*Beckman Coulter*)

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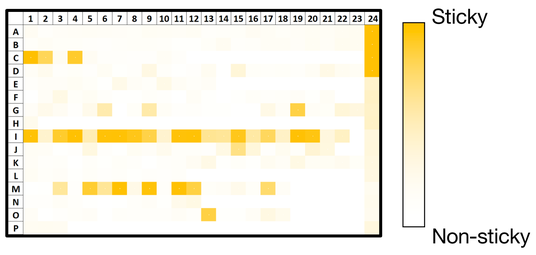
A 384-pod automated workstation used at IPI for PSR ELISA, capture ELISA, and cell display titrations



PSR ELISA Result

The shades of yellow correlate to the stickiness of the antibody candidates in this plate

Antibody Quality Control

Size-Exclusion Chromatography (SEC)

- **Daily throughput:**
  Two 96-well plates
- **Assay:**
  HPLC-driven analytical SEC that correlates protein size to retention time
- **Purpose:**
  To check the monodispersity of produced antibody candidates
- **Instrument:**
  1260 Infinity II (*Agilent*)

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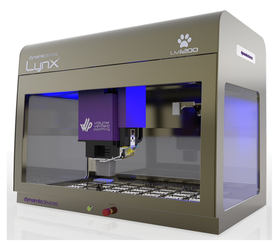
Analytical-scale LC system used at IPI for antibody validations and antigen expression evaluations.

Antibody Characterization

Bio-Layer Interferometry (BLI)

- **Daily throughput:**
  3-12 BLI 384-well plates, with 96 antibodies per plate
- **Assay:**
  High-throughput biophysical characterization of antibodies
- **Purpose:**
  To quickly identify potent antibody candidates
- **Automated workstation:**
  Lynx 96VVP (*Dynamic Devices*)
- **Instrument:**
  Octet BLI (*Sartorius*)

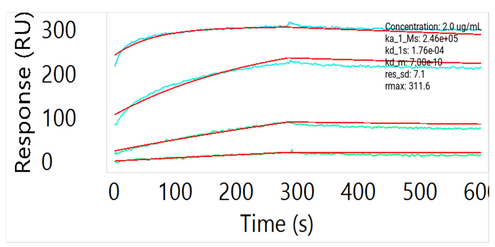


Lynx 96VVP (*Dynamic Devices*)

Very powerful automated workstation widely used at IPI for plate rearrangements, normalizations, serial dilutions, etc.

Surface plasmon resonance (SPR)

- **Daily throughput:**
  One 384-well print
- **Assay:**
  High-throughput biophysical characterization of antibodies
- **Purpose:**
  To evaluate the binding kinetics and identify the strongest binders
- **Instrument:**
  LSA (*Carterra*)



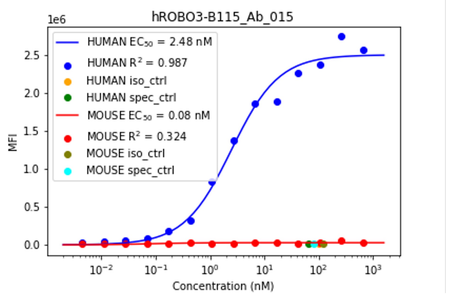
SPR Result Example

This is a potent binder (TAB00010565) against purified human ROBO3 antigen

Antibody Characterization

Cell Display

- **Daily throughput:**
  Two 384-well plates, including 48 antibodies with 14-point titrations
- **Assay:**
  High-throughput cell-staining assay using transiently transfected ExpiCHO cells
- **Purpose:**
  To examine if the antibodies can recognize membrane-bound, native-like antigens
- **Automated workstation:**
  Lynx 96VVP (*Dynamic Devices*), Biomek NX^{P} (*Beckman Coulter*)
- **Instrument:**
  iQue Screener PLUS (*Sartorius*)



Cell Display Result Example

The biophysically characterized binder (TAB00010565) also showed strong affinity toward cell-displayed, full-length human ROBO3 antigen but not to its mouse homolog.

Conclusion

IPI strives to discover and deliver consistent high-quality antibodies by using robotic liquid handling platforms. We also seek to improve platform efficiency through system optimization and integration.

Want To Collaborate With Us?

We have generated antibodies to more than 200 cell surface receptor targets.
We are open to collaborations from industry and academia.
Contact us at **https://proteininnovation.org/receptor-engineering/** or via **rob.meijers@proteininnovation.org**

Scan to connect with IPI Receptor Engineering