Establishing a Platform Ligand-Binding Method on the ForteBio Octet

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Who is KBI

• **About KBI: A Contract Development and Manufacturing Company**

• KBI helps partners accelerate and optimize drug development and manufacturing programs with an extensive suite of expert development and manufacturing services in an agile, client-friendly partnering environment
Role of Analytical Development

• Development
  • Method Development
  • Method Optimization
  • PD support (CLD, Upstream and Downstream)
  • Lots of Samples!

• Quality Control
  • Method Qualification
  • Stricter Requirements
  • Fewer Samples

Challenge: Technology that can be utilized in a Q.C. and in a high-throughput fashion

Answer: Platform Method allows for rapid development, optimization, and PD Support
How We Use Our Octet

- **ProA Titer**
  - >400 Titer samples/week

- **Kinetics/Dose Response**
  - Developed 10+ assays
  - Including Fc Receptor Panel

- **Advanced Titer**
  - Developed 5+

- **Relative Potency**
  - 9 Methods Developed
  - 6 Qualified
  - Also used as ID Test

- **Dual-Target Relative Potency**
  - Qualified Method for Dual-binding of Fc-fusion Antibody
1. Platform Development Consideration

2. Streamlining Development Using the Platform Method

3. When Platform Doesn’t Work?

4. Dual-Binding Potency Assay/ Troubleshooting
Step Times

Baseline: 30 – 60 sec.
Loading: ~ 3-5 minutes
Baseline/Wash: 60 – 120 sec.
Association: 2 – 10+ minutes

Platform:
- 60 sec.
- 5 minutes
- 60 sec.
- 5 minutes
Octet Sensor Selection

- **Ni (NTA)** Platform/Preferred
  - Protein A
  - SSA (Super Streptavidin)
  - AHC (Anti-Human Fc Capture)
  - AMC (Anti-Mouse Fc Capture)

- Anti-Human IgG Quantitation
- Aminopropylsilane
- Amine Reactive
- Anti-Human Fab
- Anti-GST

- **Anti-Penta-HIS**
- **Anti-His**
- **Protein G**
- **Protein L**
- **Streptavidin**
- **High Precision Streptavidin**

- **Kits:**
  - Anti-CHO HCP detection kit
  - Residual Protein A detection kit
  - Immunogenicity Assay Kit
Sensor Loading Impact

Protein A Sensors

Platform: ~1 nm shift at 5 minutes
Overview

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1. Load Density Scouting

- Sensor loading is evaluated using an antigen/protein to ensure sufficient protein is loaded on the sensor, but not overloaded.
2. Concentration scouting of 2\textsuperscript{nd} molecule (antibody)

- Holding the first molecule (antigen) constant, a concentration range is evaluated for product association in a concentration dependent manor.

For typical antibodies, assay development would be done => method optimization and assessment (accuracy, linearity, etc.)
Examples:

• Biosimilar Antibody/Antigen:
  • Development/ Feasibility Time => 1 Day

• FcγR Potency Assay:
  • Development/ Feasibility Time => 12 Hours (recorded by analyst)

• FcRN Kinetics: (pH Challenges)
  • Development/ Feasibility Time => 5-7 Days
• Qualified 6 single target potency methods using different antibody/antigen pairs

• Platform has been leveraged for the development and/or qualification of multiple (15+) non-potency assays (Titer, Kinetics, etc.)

• Frequently achieve ≤10% RSD for sample triplicates and 90 – 110% Potency

• Release/stability method for activity much faster than typical ELISA or SPR

\[ R^2 \geq 0.99 \]
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Common Platform Challenges

• Not all molecules can have a His-tag (no Ni-NTA sensors)

• Molecule specific challenges
  • Not stable in typical assay buffer
  • Buffer interference from ‘dirty’ samples
  • Method is not typical Antibody/Antigen
    » Glycoprotein (aggregation)
    » Bi-specific or Fusion protein (Dual Target)
Always Check Your Curves

Form 1 25 µg/mL
Form 1 12.5 µg/mL
Form 1 6.25 µg/mL
Form 2 20.3 µg/mL
Form 3 15.9 µg/mL
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An Example of When Nothing Works!

All Steps Aligned by step Baseline (Sensor Location)

Time (s)

nm
3. Addition of 3\textsuperscript{rd} molecule

- Evaluate the 3\textsuperscript{rd} molecule at multiple concentrations and identify the concentration that produces the largest wavelength shift \textit{but} keeps concentration dependent signal from previous step
Addition of 3rd Molecule
Troubleshooting

- Molecule Order
  - Switched 1\textsuperscript{st} and 3\textsuperscript{rd} Molecule
3 Molecule Troubleshooting

• Sensors:
  • Ni (NTA), Fc Capture, Streptavidin

• 1\text{\textsuperscript{st}} and 3\text{\textsuperscript{rd}} Molecules (antigens)
  • His-Tagged and/or Biotinylated of the following
  • Full Length Molecule (Transmembrane)
  • Extracellular Domain only
  • Partial Sequence (ECD and part of Transmembrane portion)
  • Non-Tagged antigens
  • Curve for the 1\text{\textsuperscript{st}} molecule (constant Ab. And 3\text{\textsuperscript{rd}})
  • Extended Ab step to saturate, then 3\text{\textsuperscript{rd}} molecule curve
  • Pre-incubating Fc Fusion with the 3\text{\textsuperscript{rd}} Molecule
3 Molecule Working?

- Sensors: Super Streptavidin
Negative Wavelength Shift

- Spectrophotometer in the instrument covers a range of 550-850 nm, a wavelength is adjusted to cover one of the cycles.

- Peak location is determined by the optical thickness \((n*d)\).

**Small** change in optical thickness vs. **Large** change in optical thickness:

- Four peaks in the window for small change.
- Five peaks in the window for large change.

Inversion of Signal

R Square 0.9964
Troubleshooting/Platform Conclusions

• Platform does not always work but can be used as a starting point

• Buffer compatibility

• Flip the assay

• Check the shapes of the curves

• Octet vs ELISA?
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