Human Monoclonal IgG Subclass Determination on the Octet QKe

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Agenda

• Introduction
• Goal
• Method development
• Conclusion
What are Monoclonal Antibodies?

- Monoclonal antibodies (mAbs) are produced from a single cell line.
- The heavy chain of monoclonal antibodies has a specific isotype subclass.
- For human IgG there are 4 subclasses: 1, 2, 3, and 4.
Why Isotyping?

• For affinity purification of mAbs from cell supernatant
  – to ensure that the correct purification method is used.

• For functional assays
  – to predict ADCC and CDC activity
Why use the Octet QKe to Isotype?

- Get results quickly: testing can be done in < 1 hour, while ELISA could take 2-3 hours;
- Less laborious compared to ELISA with multiple steps;
- The results are easy to interpret: either there is or isn’t an isotype subclass present (yes/no response).
Goal

• To establish a method for isotyping of human Ig antibodies
  – High sensitivity level (~1 μg/mL)
  – Re-usable sensor tips
  – Works well in FBS-containing cell media supernatant
Sample Plate Setup

Basic Procedure
1) Capture/Immobilize Mouse anti-human IgG mAbs on sensor tip
2) Dip into wells containing isotype-specific control mAbs
   2a) Dip into wells containing 2° detection mAb
3) Dip into wells containing unknown mAbs
   3a) Dip into wells containing 2° detection mAb
4) Regeneration/Wash
hIgG1

Sensor tip

Mouse-anti hIgG1

hIgG1

Sensor tip

Mouse-anti hIgG2

hIgG1

Sensor tip

Mouse-anti hIgG3

hIgG1

Sensor tip

Mouse-anti hIgG4

hIgG1

hIgG2,3,4

Step selected: Association (Sensor Location, Well Location)

Time (sec)

A4, A3
B4, B3
C4, C3
D4, D3
Method Development

- Three methods
  - Anti-mouse IgG-Fc tips
  - Amine reactive
  - Streptavidin
Anti-mIgG-Fc Sensor Tips

• These sensor tips bind directly to the capture mAbs, but not to the human control or sample mAbs (no cross-reactivity).

• They can also be easily and frequently regenerated.
Anti-mIgG-Fc Sensor Tips

1) Capture mouse anti-human IgG-specific mAbs.
2) Dip sensor tip directly to control or unknown.
3) Dip sensor tip directly into anti-human Fc to enhance binding signal.
4) Wash and regenerate the sensor tips and repeat.
Anti-mIgG-Fc Sensor Tips

Sensor tip
Mouse
Anti-hIgG1
Anti-mIgG-Fc Sensor Tips

Only hIgG1 mAb binds to Anti-hIgG1 capture mAb
Anti-mIgG-Fc Sensor Tips

Detection mAb only binds if human IgG is bound, since capture mAbs have mouse Fc
Anti-mIgG-Fc Sensor Tips

<table>
<thead>
<tr>
<th>Control mAb</th>
<th>Sensitivity</th>
</tr>
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<tbody>
<tr>
<td>hIgG1</td>
<td>0.5 µg/mL</td>
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<tr>
<td>hIgG2</td>
<td>5 µg/mL</td>
</tr>
<tr>
<td>hIgG3</td>
<td>2 µg/mL</td>
</tr>
<tr>
<td>hIgG4</td>
<td>0.5 µg/mL</td>
</tr>
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Control hIgG2 and hIgG3 could not be detected at as low a concentration as hIgG1 and hIgG4, even with the use of Anti-human IgG Fc 2° Antibody.
Anti-mIgG-Fc Sensor Tips

• The sensitivity was not optimal, as concentrations < 5 µg/mL for hIgG2 and < 2 µg/mL hIgG3 could not be reliably detected

• Secondary mAbs only enhanced the already strong hIgG1 and hIgG4, and not the weaker hIgG2 and hIgG3
Amine Reactive (AR2G) Sensor Tips

- Amine reactive sensor tips used to directly immobilize anti-hIgG-specific mAbs
- The sensor tips can also be easily and frequently regenerated
AR2G Sensor Tips

1) Immobilize anti-human IgG-specific mAbs

2) Establish baseline with blank buffer (PBS)

3) Dip sensor tips into control or unknown (association) followed by PBS (dissociation)

4) Wash and regenerate and repeat steps 2 and 3.
AR2G Sensor Tips

- AR2G sensor tips could not reliably detect control samples at 50 μg/mL.
Streptavidin (SA) Sensor Tips

• To remedy the issues presented with Anti-mIgG-Fc and AR2G sensor tips, switched to SA sensor tips.
1) Biotinylate Mouse Anti-human IgG-specific mAbs
2) Capture biotinylated mAb to SA sensor tip
3) Establish baseline with PBS
4) Dip sensor tips into control or unknown (association), followed by PBS (dissociation)
5) Wash and regenerate and repeat steps 3 and 4

Streptavidin Sensor Tip

Capture mAbs: Biotinylated mouse-anti-human Ig isotype-specific antibody (anti-hlgG1, hlgG2, hlgG3, or hlgG4)
SA Sensor Tips

Capture     hIgG1       hIgG2         hIgG3        hIgG4          mAb1         mAb2         Blank

Graph showing the time course of binding events for different samples over time.
## Conclusion

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<th>Streptavidin</th>
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<tr>
<td>hIgG4</td>
<td>0.5 μg/mL</td>
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</tbody>
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Regeneration: Anti-mIgG-Fc sensor tips can be used for at least two sample plates, while the SA sensor tips can be used for up to three samples plates in either PBS or FBS-containing media. SA sensor tips were shown to have the best sensitivity and reliability compared to other methods.
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Supplemental Data – Octet Isotyping
mIgG-Fc Sensor tips with anti-hIgG1 capture and 0.5 μg/mL hIgG1 control

mIgG-Fc Sensor tips with anti-hIgG2 capture and 5 μg/mL hIgG2 control
mIgG-Fc Sensor tips with anti-hIgG3 capture and 2 μg/mL hIgG3 control

mIgG-Fc Sensor tips with anti-hIgG4 capture and 0.5 μg/mL hIgG4 control
# AR2G Sensor Tips

<table>
<thead>
<tr>
<th></th>
<th>hIgG1</th>
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<th>hIgG3</th>
<th>hIgG4</th>
<th>hIgM</th>
<th>mAb1</th>
<th>mAb2</th>
<th>mAb3</th>
<th>Control</th>
<th>Unknowns</th>
</tr>
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![Graph showing sensor locations and time in seconds](image)
AR2G Immobilization
SA Sensor Tips