Antibody cross-reactivity testing against thousands of human proteins

A quantum leap in antibody cross-reactivity analysis offered as a service by CDI Laboratories.

Using its HuProt™ Human Proteome Microarray, CDI will analyze an antibody against the world’s largest collection of human proteins – both native and denatured – and at two working concentrations. The data will span ~75% of the human proteome. And we’ll deliver a detailed cross-reactivity summary in about 2 weeks.

The benefits of highly-characterized reagents - including antibodies - to users and producers are clear and cannot be overstated:

- Persuasive proposals → Fund regularly.
- Robust research → Publish faster.
- Diagnostics / therapeutics → Develop earlier; cut costs.
- Competitive advantage → Commercialize smarter.

Consider the data >>

www.cdi-lab.com
An overview of CDI’s HuProt™ human proteome microarray

The HuProt human proteome microarray provides the largest number of unique human proteins known to be included on a single slide, allowing thousands of interactions to be profiled in high-throughput - including 

antibody cross-reactivity analysis.

The HuProt™ human proteome microarray v2.0 contains thousands of unique, individually purified human proteins along with approximately 100 mouse proteins. The content encompasses ~75% of the human proteome; see table, left ) and ~100 unique mouse gene symbols. Recombinant proteins are expressed in yeast (S. cerevisiae), purified and printed on glass slides in duplicate, along with control proteins.

Using HuProt™ to analyze antibody cross-reactivity

Microarray Analysis: Antibody specificity was evaluated using CDI HuProt Human Proteome Microarray (~75% of the human proteome). The microarray is incubated with the primary antibody, rinsed, incubated with a secondary antibody and subsequently analyzed with GenePix Pro Image Acquisition and Analysis Software, the benchmark tool for the acquisition and analysis of microarray images. The top 3 “hits” are identified by cross-reference to the array map which stores the exact location of each protein. If the expected target is ranked #1 and the S-Score (the difference between Rank #1 and #2) is >3, then the antibody is considered monospecific.

Statistical Analysis: Thousands of GenePix data points (from the microarray) are analyzed in terms of signal strength and ranked accordingly.

**SUMMARY:** The A-score indicates the number of standard deviations above background seen for the mean signal bound by the target antigen. The S-score represents the difference between the A-score of the target antigen and the next best hit on the array.

S-scores greater than 3 standard deviations over the next listed target are deemed statistically significant and indicate highly specific antibodies.

When S > 3 over the next listed target, it is indicative of a monospecific antibody.
Consider the data generated using commercially available antibodies:

**A monospecific, high-quality PCNA antibody**

![Expected Target](image1)

**A cross-reactive, low-quality p53 antibody**

![Expected Target](image2)

**Ab Cross-reactivity vs. working concentration (Anti-PCNA)**

![A-Score](image3)

**Ab Cross-reactivity: native vs. denatured proteins (Anti-p53)**

**Statistically Insignificant Cross-reactivity**

**Expected Target, monospecific**

**Unexpected Target**

**Ab Cross-reactivity: 0.1 µg/ml**

**Ab Cross-reactivity: 1.0 µg/ml**

**Expected Target**

**Unexpected Target**

**Note:** At 1 µg/ml, this antibody is considered monospecific for OTUB1.

**Note:** On the native array, this antibody is considered monospecific for C20orf77.

See more data summaries using other commercial antibodies:

www.cdi-lab.com     844-539-6296 TOLL FREE US/Canada  © CDI Laboratories, Inc.
Cross-reactivity analysis of other commercial antibodies: summarized data

### ELISA Confirmation

<table>
<thead>
<tr>
<th>Company</th>
<th>Intended Target (IT)</th>
<th>Dilution µg/ml</th>
<th>Top Target</th>
<th>A Score</th>
<th>S Score*</th>
<th>Specific for IT</th>
<th>Array*</th>
<th>White Paper**</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company C</td>
<td>BCL2</td>
<td>0.1</td>
<td>AMPH</td>
<td>28.94</td>
<td>1.75</td>
<td>NO</td>
<td>N</td>
<td>p. 5</td>
<td>ELISA confirmation of HuProt™ analysis; each antibody is non-specific for the intended target (IT)</td>
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<tr>
<td>Company A</td>
<td>USF2</td>
<td>1.0</td>
<td>USF2</td>
<td>31.26</td>
<td>0.65</td>
<td>NO</td>
<td>N</td>
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<td></td>
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<tr>
<td>Company G</td>
<td>KCNP2</td>
<td>1.0</td>
<td>RBKS</td>
<td>56.58</td>
<td>0.00</td>
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<td>N</td>
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### Native Microarray

<table>
<thead>
<tr>
<th>Company</th>
<th>Intended Target (IT)</th>
<th>Dilution µg/ml</th>
<th>Top Target</th>
<th>A Score</th>
<th>S Score*</th>
<th>Specific for IT</th>
<th>Array*</th>
<th>White Paper**</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company C</td>
<td>PTEN</td>
<td>1.0</td>
<td>PTEN</td>
<td>69.31</td>
<td>1.60</td>
<td>NO</td>
<td>N</td>
<td>p. 9</td>
<td>head-to-head comparison; Biologend PTEN Ab is also monospecific on denatured array (see below)</td>
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<tr>
<td>Company B</td>
<td>PTEN</td>
<td>0.1</td>
<td>ZNF175</td>
<td>6.53</td>
<td>0.27</td>
<td>NO</td>
<td>N</td>
<td>p. 10</td>
<td>monospecific for unintended target at higher concentration</td>
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<tr>
<td>Company C</td>
<td>PCNA</td>
<td>0.1</td>
<td>PCNA</td>
<td>111.70</td>
<td>86.83</td>
<td>YES</td>
<td>N</td>
<td>p. 11-12</td>
<td>loss of monospecificity at higher concentration; same results on denatured array (see below)</td>
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<tr>
<td>Company C</td>
<td>PCNA</td>
<td>0.1</td>
<td>OTUB1</td>
<td>74.59</td>
<td>7.66</td>
<td>NO</td>
<td>N</td>
<td>p. 11-12</td>
<td>monospecific for unintended target at higher concentration</td>
</tr>
<tr>
<td>Company D</td>
<td>PCNA</td>
<td>1.0</td>
<td>FMN1</td>
<td>8.39</td>
<td>0.76</td>
<td>NO</td>
<td>N</td>
<td>p. 13</td>
<td>no specificity at either concentration</td>
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<tr>
<td>Company C</td>
<td>TP53</td>
<td>0.1</td>
<td>C20orf77</td>
<td>7.22</td>
<td>2.03</td>
<td>NO</td>
<td>N</td>
<td>p. 14-15</td>
<td>no specificity at either concentration</td>
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<tr>
<td>Company F</td>
<td>TP53</td>
<td>0.1</td>
<td>C20orf77</td>
<td>57.86</td>
<td>9.55</td>
<td>NO</td>
<td>N</td>
<td>p. 14-15</td>
<td>monospecific for unintended target at lower concentration; monospecific for intended target at higher concentration</td>
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</table>

### Denatured Microarray

<table>
<thead>
<tr>
<th>Company</th>
<th>Intended Target (IT)</th>
<th>Dilution µg/ml</th>
<th>Top Target</th>
<th>A Score</th>
<th>S Score*</th>
<th>Specific for IT</th>
<th>Array*</th>
<th>White Paper**</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company C</td>
<td>PTEN</td>
<td>0.1</td>
<td>PTEN</td>
<td>29.83</td>
<td>0.00</td>
<td>NO</td>
<td>D</td>
<td>p. 16</td>
<td>no specificity on either array</td>
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<tr>
<td>Company B</td>
<td>PTEN</td>
<td>0.1</td>
<td>PTEN</td>
<td>52.50</td>
<td>22.08</td>
<td>YES</td>
<td>D</td>
<td>p. 17</td>
<td>high-quality antibody as demonstrated on both native and denatured arrays</td>
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<tr>
<td>Company D</td>
<td>PTEN</td>
<td>1.0</td>
<td>ZNF490</td>
<td>12.29</td>
<td>0.53</td>
<td>NO</td>
<td>D</td>
<td>p. 18</td>
<td>monospecific for unintended target at lower concentration</td>
</tr>
<tr>
<td>Company C</td>
<td>PCNA</td>
<td>0.1</td>
<td>PCNA</td>
<td>125.93</td>
<td>86.38</td>
<td>YES</td>
<td>D</td>
<td>p. 19</td>
<td>high-quality antibody as demonstrated on a denatured array; use with caution on native proteins (see above)</td>
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<tr>
<td>Company E</td>
<td>PCNA</td>
<td>0.1</td>
<td>ZNF639</td>
<td>29.46</td>
<td>0.00</td>
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<td>p. 20</td>
<td>loss of monospecificity at higher concentration; same results on native array (see above)</td>
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<tr>
<td>Company C</td>
<td>TP53</td>
<td>0.1</td>
<td>FAM114A1</td>
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<td>1.86</td>
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<td>D</td>
<td>p. 21</td>
<td>no specificity on either array</td>
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<tr>
<td>Company F</td>
<td>TP53</td>
<td>0.1</td>
<td>EP400NL</td>
<td>21.38</td>
<td>1.86</td>
<td>NO</td>
<td>D</td>
<td>p. 22</td>
<td>high-quality antibody as demonstrated on a denatured array; use with caution on native proteins (see above)</td>
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</tbody>
</table>

* S Scores greater than or equal to 3.00 indicate a monospecific antibody and are indicated in bold.

Note that 4 antibodies demonstrate monospecificity for UNINTENDED TARGETS under certain conditions.

**Download the new white paper: *Antibody Cross-Reactivity Testing Using the HuProt™ Human Proteome Microarray***

Give us a call for more information, to get a quote or to begin a project.

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