Introduction
α-crystallins composed of ~20 kDa αA and αB subunits function as major water-soluble proteins accounting for almost 50% of total protein in the mammalian transparent eye lens, also existing in a variety of other tissues. The α-crystallin proteins are members of the small heat shock protein (sHsp) family, as their expression can be induced by heat and other stress insults in a variety of organisms. The α-crystallins possess structural and functional similarities and share sequence homology with Hsp25/27. The conserved α-crystallin domain participates in oligomer assembly, which is critical to their function in the prevention of irreversible protein aggregation.

Materials Provided
1. α-B Crystallin Capture Antibody
2. α-B Crystallin Standard
3. α-B Crystallin Detection Antibody
4. SA-HRP

Materials Needed but not Supplied
1. RIPA Cell Lysis Buffer, Cat. #80-1284, or similar
2. 96-well high-binding polystyrene microtiter plates, Cat. #80-1930, or similar
3. Precision pipets
4. Microplate reader capable of reading at 450 nm
5. Microplate shaker
6. Phosphate buffered saline (PBS)*
7. Tween®20*
8. Bovine Serum Albumin (BSA)*
9. 3,3’,5,5’ tetramethylbenzidine (TMB) solution, Cat. #80-1930 or similar
10. 1N hydrochloric acid, such as Stop Solution 2, Cat. #80-1804
11. Sucrose

Buffer Formulations
1. Coating Buffer
   - 10 mM sodium phosphate, 15 mM NaCl, pH 7.4
2. Blocking Buffer
   - 10 mM sodium phosphate, 15 mM NaCl, 1.0% BSA, 1.0% sucrose, pH 7.4
3. Assay Buffer
   - 100 mM sodium phosphate, 150 mM NaCl, 1.0% BSA, 0.1% Tween-20, pH 7.4
4. Wash Buffer
   - 10 mM sodium phosphate, 15 mM NaCl, 0.1% Tween-20, pH 7.4

Plate Coating
1. Reconstitute α-B Crystallin Capture Antibody with 500 µg lyophilized α-B Crystallin protein, Cat. #80-1935
2. Dilute the stock 1:250 in Coating Buffer. Immediately dispense into 96-well microtiter plates. Use immediately, or make aliquots and freeze at -20°C. Avoid repeated freeze/thaw cycles.

Plate Preparation
1. Reconstitute α-B Crystallin Standard with 250 µL deionized water for a 50x stock. Store at -4°C for up to 3 months, or aliquot and freeze at -20°C for prolonged storage. Avoid repeated freeze/thaw cycles.
3. SA-HRP
Reconstitute vials with 250 µL deionized water for a 50x stock. Store at -4°C for up to 3 months, or aliquot and freeze at -20°C for prolonged storage. Avoid repeated freeze/thaw cycles.

Assay Procedure
1. Pipet 100 µL of Assay Buffer into the control (0 ng/mL standard) wells.
2. Pipet 100 µL of standards and samples, prepared in Assay Buffer, to the bottom of the appropriate wells.
3. Seal the plate. Incubate for 1 hour on a plate shaker at room temperature.
4. Empty the contents of the wells and wash by adding 400 µL of Wash Buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
5. Pipet 100 µL of the diluted detection antibody into each well, except the blank.
6. Seal the plate. Incubate for 1 hour on a plate shaker at room temperature.
7. Wash as above (Step 4).
8. Add 100 µL of the diluted conjugate to each well except the blank.
9. Seal the plate. Incubate for 30 minutes on a plate shaker at room temperature.
10. Wash as above (Step 4).
11. Pipet 100 µL of TMB solution into each well.
12. Seal the plate. Incubate for 30 minutes on a plate shaker at room temperature.
13. Pipet 100 µL 1 N HCl into each well.
14. After blanking the plate reader against the substrate, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Assay Performance

Typical Data
The results shown below are for illustration only and should not be used to interpret results from another assay.

Dilutional Linearity
To determine possible interference from the sample matrix, the indicated sample types were serially diluted into assay buffer. The concentrations of αB-Crystallin were measured in the assay, and the results were analyzed to determine the range over which a linear response was obtained. These data may be used as a guideline to determine minimal recommended dilution (MRD) for similar samples.

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>HeLa CL</th>
<th>C6 CL</th>
<th>3T3 CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>32%</td>
<td>31%</td>
<td>40%</td>
</tr>
<tr>
<td>1:4</td>
<td>56%</td>
<td>67%</td>
<td>77%</td>
</tr>
<tr>
<td>1:8</td>
<td>92%</td>
<td>107%</td>
<td>101%</td>
</tr>
<tr>
<td>1:16</td>
<td>80%</td>
<td>103%</td>
<td>100%</td>
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<tr>
<td>1:32</td>
<td>100%</td>
<td>92%</td>
<td>---</td>
</tr>
<tr>
<td>1:64</td>
<td>---</td>
<td>100%</td>
<td>---</td>
</tr>
</tbody>
</table>

CL: Cell Lysate

Parallelism
Dose-response curves from cell lysates diluted into assay buffer (using the MRD) were compared to the recombinant αB-Crystallin standard curve. Parallelism indicates that the antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of the analyte.

Sensitivity
The sensitivity, or limit of detection, of this assay is 0.59 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 5 standard curves.

Specificity
This assay detects human, mouse, rat, and bovine αB-Crystallin. Cross reactivity with γ-Crystallin and αA-Crystallin is 0.06% and 0.4%, respectively. There is no cross reactivity observed with β-L-Crystallin, Hsp10, Hsp25 and Hsp27.

Calculation of Results
Several options are available for the calculation of the relative levels of αB-Crystallin in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve-fitting program. For accuracy, please ensure that sample values fall within the standard range.

<table>
<thead>
<tr>
<th>Accessory Reagent List</th>
<th>Reagent</th>
<th>Quantity</th>
<th>Cat. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmunoSet® Buffer Pack</td>
<td>1 each of the following products: 80-1927, 80-1928, 80-1929, 80-1805, 80-1804</td>
<td>ADI-950-003</td>
<td></td>
</tr>
<tr>
<td>ImmunoSet® Plate Pack</td>
<td>5 96-well microtiter plates &amp; 5 plate sealers</td>
<td>80-1930</td>
<td></td>
</tr>
<tr>
<td>PBS Concentrate</td>
<td>120 mL</td>
<td>80-1927</td>
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<tr>
<td>BSA Solution (10%)</td>
<td>50 mL</td>
<td>80-1928</td>
<td></td>
</tr>
<tr>
<td>Tween-20 Solution (10%)</td>
<td>30 mL</td>
<td>80-1929</td>
<td></td>
</tr>
<tr>
<td>RIPA Cell Lysis Buffer 2</td>
<td>100 mL</td>
<td>80-1284</td>
<td></td>
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<tr>
<td>Wash Buffer Concentrate</td>
<td>100 mL</td>
<td>80-1287</td>
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<tr>
<td>SA-HRP</td>
<td>12.5 µg/vial</td>
<td>80-1896</td>
<td></td>
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</tbody>
</table>

Storage
Store all components at 4°C. See page 3 for storage of reconstituted material.

Tips & Troubleshooting
- If buffers other than those recommended are used in the assay, the end-user must determine the appropriate dilution and assay validation.
- Pipet the reagents to the sides of the wells to avoid possible contamination.
- Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.
- Insufficient washing or residual wash buffer in the wells may cause variation in assay results.
- Bring all reagents to room temperature for at least 30 minutes prior to opening.
- All standards, controls, and samples should be assayed in duplicate.

Limited Warranty
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