Introduction and Background

A. Overview
Albumin is serum hepatic protein, the most abundant protein in serum and contributes to the maintenance of oncotic pressure as well as to transport of hydrophobic molecules (1). Serum albumin level has been linked in clinical practice to several diseases. Low albumin levels can suggest liver (2), kidneys disease (3), inflammation (4), shock (5), and malnutrition (6). On the other hand, high albumin levels usually reflect dehydration (7).

B. Test Principle
The kit employs a quantitative competitive sandwich enzyme immunoassay technique that measures albumin in rat plasma and serum in less than 2 hours. A polyclonal antibody specific for rat albumin has been pre-coated onto a 96-well microplate with removable strips. Albumin in standards and samples is competed by a biotinylated Albumin sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

C. Notice for Application of Kit
✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-protein, and SP conjugate) as instructed, prior to running the assay.
✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
✓ Spin down the SP conjugate vial before opening and using contents.
✓ This kit is for research use only.
✓ The kit should not be used beyond the expiration date.
✓ The Stop Solution is an acid solution.

Material and Method

A. List of component
1. Rat Albumin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat albumin.
2. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
5. MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml).
7. **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
8. **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
9. **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

**B. Additional Required Materials But Not Provided**
1. Microplate reader capable of measuring absorbance at 450 nm
2. Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel)
3. Deionized or distilled reagent grade water

**C. Sample Collection, Preparation and Storage**
- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:8000 into MIX Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:8000 into MIX Diluent. Store serum at -20°C or below. Avoid repeated freeze-thaw cycles.

**D. Reagent Preparation**
- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Standard Curve:** Reconstitute the 150 µg of albumin standard with 1.5 ml of MIX Diluent to generate a solution of 100 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (100 µg/ml) 1:2 with MIX Diluent to generate 50, 25, 12.5, 6.25, 3.13 and 1.56 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C.

<table>
<thead>
<tr>
<th>Standard Point</th>
<th>Dilution</th>
<th>[rAlbumin] (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Standard (100 µg/ml)</td>
<td>100.00</td>
</tr>
<tr>
<td>P2</td>
<td>1 part P1 + 1 parts MIX Diluent</td>
<td>50.00</td>
</tr>
<tr>
<td>P3</td>
<td>1 part P2 + 1 parts MIX Diluent</td>
<td>25.00</td>
</tr>
<tr>
<td>P4</td>
<td>1 part P3 + 1 parts MIX Diluent</td>
<td>12.50</td>
</tr>
<tr>
<td>P5</td>
<td>1 part P4 + 1 parts MIX Diluent</td>
<td>6.25</td>
</tr>
<tr>
<td>P6</td>
<td>1 part P5 + 1 parts MIX Diluent</td>
<td>3.13</td>
</tr>
<tr>
<td>P7</td>
<td>1 part P6 + 1 parts MIX Diluent</td>
<td>1.56</td>
</tr>
<tr>
<td>P8</td>
<td>MIX Diluent</td>
<td>0.00</td>
</tr>
</tbody>
</table>
- **Biotinylated Albumin (2x):** Dilute Biotinylated Albumin with 5 ml MIX Diluent to produce a 2-fold stock solution, which can be further diluted 1:2 with MIX Diluent. Any remaining solution should be frozen at -20°C.

- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.

- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

### E. Stability and storage

1. Store kit at 2-8°C or -20°C upon arrival up to the expiration date.
2. Opened MIX Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below.
3. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

### F. Protocol

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 25 µl of standard or sample per well, and immediately add 25 µl of Biotinylated Albumin to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for one hour. Start the timer after the last sample addition.
4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
5. Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash a microplate as described above.
7. Add 50 µl of Chromogen Substrate per well and incubate for about 7 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
8. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
9. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generates at high concentration points after stopping the reaction for about 10 minutes, which will reduce
Result

A. Data analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

B. Standard Curve

![Rat Albumin Standard Curve](image)

This standard curve was generated for demonstration purpose only. **A standard curve must be run with each assay.**

C. Performance Characteristics

- The minimum detectable dose of Albumin is typically 1 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.3% respectively.

D. Linearity

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Plasma</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:4000</td>
<td>99%</td>
<td>98%</td>
</tr>
<tr>
<td>1:8000</td>
<td>102%</td>
<td>99%</td>
</tr>
<tr>
<td>1:16000</td>
<td>103%</td>
<td>101%</td>
</tr>
</tbody>
</table>
E. Recovery

<table>
<thead>
<tr>
<th>Standard Added Value</th>
<th>2-20 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery %</td>
<td>85-113 %</td>
</tr>
<tr>
<td>Average Recovery %</td>
<td>101 %</td>
</tr>
</tbody>
</table>

F. Cross-Reactivity

<table>
<thead>
<tr>
<th>Species</th>
<th>% Cross Reactivity</th>
</tr>
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<tbody>
<tr>
<td>Human</td>
<td>&gt; 0.1%</td>
</tr>
<tr>
<td>Mouse</td>
<td>&gt; 0.1%</td>
</tr>
<tr>
<td>Rat</td>
<td>100%</td>
</tr>
<tr>
<td>Swine</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Bovine</td>
<td>&gt; 0.1%</td>
</tr>
<tr>
<td>Beagle</td>
<td>&gt; 0.1%</td>
</tr>
<tr>
<td>Monkey</td>
<td>&gt; 0.1%</td>
</tr>
</tbody>
</table>

References