### Human Serum Albumin Assay

**Immunoenzymetric Assay for the Measurement of Human Serum Albumin**  
**Catalog # F055**

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#### Intended Use

This kit is intended for use in quantitating human albumin. The kit is for Research and Manufacturing use only and is not intended for diagnostic use in humans or animals.

#### Summary and Explanation Of the Test

The manufacture of products by various biotechnological processes such as cell or tissue culture can result in residual contamination of the desired product by components used in the culture media. The use of so called serum free defined media greatly reduces the number of potential contaminants but it may still be necessary to determine trace contamination levels of the proteins and growth factors used in these media. Most commercial formulations of serum free media contain significant amounts of albumin and transferrin usually of either human or bovine origin, and insulin from various species. When the intended product may be used as a therapeutic agent in humans or animals the product should be highly purified to avoid potential health risks or other problems which might result from trace contaminants. Efforts to reduce trace media contamination to the lowest levels practical through optimal process design, validation, and final product testing require a highly sensitive and reliable analytical method. The Cygnus Technologies Human Serum Albumin assay is designed to provide a simple to use, precise, and highly sensitive method to detect human Albumin to less than 200pg/mL. As such this kit can be used as a tool to aid in optimal purification process development and in routine quality control of in-process streams as well as final product.

#### Principle of the Procedure

The human albumin assay is a two-site immunoenzymetric assay using an amplified biotin/streptavidin detection system for maximum sensitivity. Samples which may contain human albumin are reacted in microtiter strips coated with an affinity purified capture antibody. A second biotinylated anti-human antibody is reacted forming a sandwich complex of solid phase antibody-albumin-biotinylated antibody. After a wash step to remove any unbound reactants the strips are then reacted with streptavidin labeled with alkaline phosphatase. Another wash sequence is performed followed by the addition of p-nitrophenyl phosphate (PNPP) substrate. The amount of hydrolyzed substrate is read on a microtiter plate reader and will be directly proportional to the concentration of human albumin present. Accurate quantitation is achieved by comparing the signal of unknowns to human albumin standards assayed at the same time.

#### Reagents & Materials Provided

<table>
<thead>
<tr>
<th>Component</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat anti-human albumin, biotinylated</td>
<td>F056</td>
</tr>
<tr>
<td>Affinity purified goat antibody in a protein matrix with preservative, 1x12mL.</td>
<td></td>
</tr>
<tr>
<td>Goat anti-albumin coated microtiter strips</td>
<td>F058</td>
</tr>
<tr>
<td>12x8 well strips in a bag with desiccant</td>
<td></td>
</tr>
<tr>
<td>Human Albumin Standards</td>
<td>F057</td>
</tr>
<tr>
<td>human Albumin in a protein matrix. Standards at 0, 0.5, 2, 5, and 20 ng/mL, 1mL/vial</td>
<td></td>
</tr>
<tr>
<td>Streptavidin:Alkaline Phosphatase</td>
<td>F009</td>
</tr>
<tr>
<td>in a protein matrix with preservative, 1x12 mL</td>
<td></td>
</tr>
<tr>
<td>PNPP Substrate</td>
<td>F008</td>
</tr>
<tr>
<td>p-nitrophenyl phosphate in a Diethanolamine buffer, with preservative, 1x12mL</td>
<td></td>
</tr>
<tr>
<td>Wash Concentrate (20X)</td>
<td>F004</td>
</tr>
<tr>
<td>Tris buffered saline with preservative, 1x50mL</td>
<td></td>
</tr>
</tbody>
</table>

#### Storage & Stability

* All reagents should be stored a 2°C to 8°C for stability until the expiration date printed.  
* The substrate reagent should not be used if its absorbance at 405nm is greater than 0.5.  
* Reconstituted wash solution is stable until the expiration date of the kit.
Materials & Equipment

Required But Not Provided

Microtiter plate reader spectrophotometer with
dual wavelength capability at 405 & 492nm
Microtiter plate washing system
Pipettors - 50μL and 100μL
Repeating or multichannel pipettor - 100μL
Microtiter plate rotator (150 - 200 rpm)
Distilled water
1 liter container for wash solution storage

Optional Equipment

Microtiter plate incubator at 37°C

Precautions

For research or manufacturing use only.
Avoid contamination of kit reagents with
equipment or work areas which have been in
contact with concentrated sources of human albumin containing products such as human serum or cell culture media. If possible use
dedicated pipettes and tips with aerosol barrier filters to minimize chances for contamination from these sources of HSA.

Preparation of Reagents

* Bring all reagents to room temperature.
* Dilute wash concentrate to 1 liter in distilled water,
and label with kit lot and expiration date and store at
4°C.

Procedural Notes

1. Complete washing of the plates to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. If duplicate CVs are poor or if the absorbance of the 0 standard minus a substrate blank is greater than 0.2 evaluate plate washing system for proper performance.

2. High Dose Hook Effect may be observed in samples with very high concentrations of albumin. Samples greater than 20μg/mL may give absorbances less than the 20ng/mL standard. If a hook effect is possible samples should also be assayed diluted. If the absorbance of the undiluted sample is less than the diluted samples this may be indicative of the hook effect. Such samples should be diluted until the dilution adjusted value remains constant. The diluent used should be compatible with accurate recovery. Prospective diluents can be tested for recovery by using them to dilute the 20ng/mL standard, as
described in the “Limitations” section below. Alternatively, sample diluent validated for this assay, Catalog # 1028, can be purchased from Cygnus Technologies.

3. If human serum products are used in the laboratory where this assay is performed it is important to guard against contamination of the various reagents in this kit. Human albumin is typically present in greater than 20mg/mL in human serum and since this kit is able to detect 200pg/mL of albumin contamination as little as 10 parts per billion HSA in the laboratory environment could cause falsely elevated levels of albumin. High backgrounds or poor duplicates CVs are suggestive of albumin contamination. It is recommended that this assay be performed in an area where human serum products are not used with equipment which has not come into contact with human serum albumin.

Limitations

* Cross reactivity of these antibodies with albumin from other species has not been extensively investigated. Use of this kit to measure human albumin in samples containing significant quantities of albumin from another species should be appropriately validated.

* Certain sample matrices may interfere in this assay. Although the assay is designed to minimize matrix interference, materials such as detergents in high concentration, extremes of pH (<5.0 and >8.5) or very high protein concentrations may give erroneous results.

* It is recommended to test the sample matrix for interference by diluting the 20ng/mL standard 1 part to 3 parts of the matrix which does not contain any human albumin. This diluted standard when assayed as an unknown should give a value of 4 to 6ng/mL. If this is not the case then standards should be made up in the actual sample matrix being tested. Consult Cygnus Technologies Technical Service Department for advice on how to quantitate the assay in problematic matrices.

Assay Protocol

* Bring all reagents to room temperature.

* Set-up plate spectrophotometer to read dual wavelength at 405nm for the test wavelength and 492nm for the reference wavelength. Blank the instrument using the zero standard wells after assay completion.

* All standards, controls and samples should be assayed in duplicate.
* Maintain a repetitive timing sequence from well to well for all assay steps to insure that all incubation times are the same for each well.
* Make a work list for each assay to identify the location of each standard control and sample.
* If the substrate has a distinct yellow color prior to the assay it may have been contaminated. If this appears to be the case read 200μL of substrate against a water blank. If the absorbance is greater than 0.5 it may be necessary to obtain new substrate or the sensitivity of the assay may be compromised.
* Thorough washing is essential to proper performance of this assay.

**Procedural Modifications**

- Sensitivity can be further improved by extending substrate incubation times. Alternatively the plate can be incubated during the substrate step at 37°C on an approved microtiter plate incubator.
- If the high concentration end of the standard curve yields absorbances off scale or above the linear range of the plate reader it may be possible to re-read the plate at an off peak wavelength such as 450nm instead of the optimal 405nm to achieve better quantitation for high concentration samples.
- Samples containing human albumin greater than 20 ng/mL should be diluted in an appropriate diluent. (See Procedural Note # 2 concerning High Dose Hook Effect). Be sure to multiply diluted sample concentrations by the dilution factor when calculating the results. Alternatively the assays’ upper range can be extended by making higher concentration standards and reducing sample size and or incubation times Contact our customer service for guidance if you routinely require quantitation above 20 ng/mL.

**Cygnus** can also provide concentrated human albumin to make your custom standards for a nominal charge.

**Calculation of Results**

The standards may be used to construct a standard curve with values reported in ng/mL. This data reduction may be performed through computer methods using curve fitting routines such as point to point, spline, or polynomial methods. Data may also be manually reduced by plotting the absorbance values of the standard on the y-axis versus concentration on the x-axis and drawing a smooth point to point line. Absorbances of samples are then interpolated from this standard curve.

**Example Data**

<table>
<thead>
<tr>
<th>Well #</th>
<th>Contents</th>
<th>Abs. at 405nm</th>
<th>Mean Abs.</th>
<th>ng/mL albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Zero Std</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1B</td>
<td>Zero Std</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>1C</td>
<td>0.5ng/mL</td>
<td>0.032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1D</td>
<td>0.5ng/mL</td>
<td>0.031</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>1E</td>
<td>2ng/mL</td>
<td>0.127</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>1F</td>
<td>2ng/mL</td>
<td>0.123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1G</td>
<td>5ng/mL</td>
<td>0.485</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1H</td>
<td>5ng/mL</td>
<td>0.495</td>
<td>0.490</td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>20ng/mL</td>
<td>1.937</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>20ng/mL</td>
<td>1.997</td>
<td>1.967</td>
<td></td>
</tr>
<tr>
<td>2C</td>
<td>sample 1</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2D</td>
<td>sample 1</td>
<td>0.010</td>
<td>0.008</td>
<td>&lt;0.2ng</td>
</tr>
<tr>
<td>2E</td>
<td>sample 2</td>
<td>0.130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2F</td>
<td>sample 2</td>
<td>0.118</td>
<td>0.124</td>
<td>1ng</td>
</tr>
</tbody>
</table>

**Performance Characteristics/Quality Control**

**Protocol**

1. Pipette 50μL of standards, controls and samples into wells indicated on work list.

2. Pipette 100μL of biotinylated anti-albumin into each well.

3. Transfer to rotator and incubate at 180rpm for 1 hour.

4. Aspirate, wash with 300μL of diluted wash solution and aspirate. Wash a total of 3 times.

5. Pipette 100μL of Streptavidin:Alkaline Phosphatase into each well.

6. Transfer to rotator and incubate at 180rpm for 1 hour.

7. Aspirate, wash with 300μL of diluted wash solution and aspirate. Wash a total of 4 times.

8. Pipette 100μL of substrate.

9. Incubate for 1 hour.

10. Read absorbance at 405/492nm blanking on the Zero standard.
- Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples greater than 1 ng/mL. CVs for samples < 1 ng/mL may be greater than 10%.
- For optimal performance the absorbance of the substrate when blanked against water should be < 0.5.
- It is recommended that each laboratory assay appropriate quality control samples in each run to insure that all reagents and procedures are correct.

Ordering Information/
Customer Service

To place an order or to obtain additional product information contact Cygnus Technologies Customer Support:

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