



AssayMax Human Albumin ELISA Kit (Cell Culture Supernatants & Urine Samples)

Catalog Number EA3201-1

Introduction

Albumin is a 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), kidney disease (3), inflammation (4), shock (5), and malnutrition (6). On the other hand, high albumin levels usually reflect dehydration (7).

Principal of the Assay

The AssayMax Human Albumin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human albumin in urine and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human albumin in less than 2.5 hours. A polyclonal antibody specific for human albumin has been pre-coated onto a 96-well microplate with removable strips. Albumin in standards and samples is sandwiched by the immobilized polyclonal antibody and biotinylated polyclonal antibody specific for human albumin, which is recognized by a 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.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Human Albumin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human albumin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Albumin Standard:** Human albumin in a buffered protein base (800 ng, lyophilized).
- **Biotinylated Human Albumin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against human albumin (80 µl).

- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C or below.
- 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.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000µl and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:1000 into MIX Diluent. Store samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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 2-8⁰C.
- **Standard Curve:** Reconstitute the 800 ng of Albumin Standard with 4 ml of MIX Diluent to generate a standard solution of 200 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (200 ng/ml) 1:2 with equal volume of MIX Diluent to produce 100, 50, 25 12.5, 6.25, and 3.125 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[H. Albumin] (ng/ml)
P1	Standard (200 ng/ml)	200.000
P2	1 part P1 + 1 part MIX Diluent	100.000
P3	1 part P2 + 1 part MIX Diluent	50.000
P4	1 part P3 + 1 part MIX Diluent	25.000
P5	1 part P4 + 1 part MIX Diluent	12.500
P6	1 part P5 + 1 part MIX Diluent	6.250
P7	1 part P6 + 1 part MIX Diluent	3.125
P8	MIX Diluent	0.000

- **Biotinylated Human Albumin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 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.

Assay Procedure

- 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).
- 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.
- Add 50 µl of standard or sample per well. Cover wells with a sealing tape and incubate for one hour. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 µl of Biotinylated Human Albumin Antibody to each well and incubate for 30 minutes.
- Wash five times with 200 µl of Wash Buffer.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.

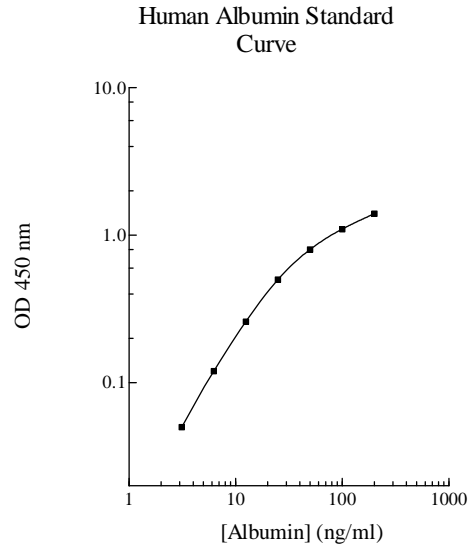
Data Analysis

- Calculate the mean value of the 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.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of albumin is typically 500 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.1% respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	Cell Culture Media (cell expressed human albumin)
1:100	100%
1:200	105%
1:400	107%

	Average Percentage of Expected Value
Sample Dilution	Urine
1:500	98%
1:1000	98%
1:2000	101%

Recovery

Standard Added Value	10 – 100 ng
Recovery %	89-111 %
Average Recovery %	100 %

Cross-Reactivity

- No significant cross-reactivity or interference was observed.

Species	% Cross Reactivity
Bovine	< 0.01
Mouse	< 0.1
Rat	< 0.1
Swine	< 1

- 10% FBS in culture media will not affect the assay.

References

- (1) Gekle M. (2004) *Ann. Rev. Physiol.*
- (2) Schindler C *et al.* (1999) *J. Hepatol.* 31(6): 1132
- (3) Hemmelder MH *et al.* (1997) *Nephrol Dial. Transplant* 12 Suppl 2:57-62
- (4) Sesmilo G *et al.* (2004) *Ann. Intern. Med.* 133(2): 111-22
- (5) Wettstein R *et al.* (2004) *Shock* 2(4): 351-357
- (6) Saito T *et al.* (1991) *Jpn. J. Surg.* 21(4): 402-11
- (7) Strand TA (2004) *Am. J. Clin. Nutr.* 79(3): 451-6

Version 4.8

Related Products

- EA2201-1 AssayMax Human Albumin ELISA Kit (for plasma/serum samples)
- EMA2201-1 AssayMax Mouse Albumin ELISA Kit (for plasma/serum samples)
- EMA3201-1 AssayMax Mouse Albumin ELISA Kit (for cell culture supernatant)
- ERA2201-1 AssayMax Rat Albumin ELISA Kit (for plasma/serum samples)
- ERA3201-1 AssayMax Rat Albumin ELISA Kit (for cell culture supernatant)
- ETA2201-1 AssayMax Rabbit Albumin ELISA Kit
- EPA3201-1 AssayMax Porcine Albumin ELISA Kit (for cell culture supernatant)