



# AssayMax Rabbit Albumin ELISA Kit

Catalog No. ETA2201-1

## Introduction

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), 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 Rabbit Albumin ELISA (Enzyme-Linked Immunosorbent Assay) kit employs a quantitative competitive enzyme immunoassay technique that measures rabbit plasma, serum, cell culture supernatant and urine albumin in less than 3 hours. A monoclonal antibody specific for rabbit 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.

## 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

- **Rabbit Albumin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against rabbit 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.
- **Rabbit Albumin Standard:** Rabbit albumin in a buffered protein base (100 µg, lyophilized).
- **Biotinylated Albumin:** 1 vial, lyophilized.
- **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 kit at 2-8<sup>0</sup>C or -20<sup>0</sup>C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8<sup>0</sup>C. Store reconstituted reagents at -20<sup>0</sup>C 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, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

## 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 1:2000 into MIX Diluent. Store samples at -20<sup>0</sup>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 1:2000 into MIX Diluent. Store serum at -20<sup>0</sup>C or below. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20<sup>0</sup>C 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 1:2 into MIX Diluent. Store samples at -20<sup>0</sup>C 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 2-8<sup>0</sup>C.
- **Standard Curve:** Reconstitute the 100 µg of Albumin Standard with 1 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 triplicate standard points by serially diluting the standard solution (100 µg/ml) 1:2 with equal volume 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<sup>0</sup>C.

Standard Point	Dilution	[hAlbumin] ( $\mu\text{g/ml}$ )
P1	Standard (100 $\mu\text{g/ml}$ )	100.00
P2	1 part P1 + 1 parts MIX Diluent	50.00
P3	1 part P2 + 1 parts MIX Diluent	25.00
P4	1 part P3 + 1 parts MIX Diluent	12.50
P5	1 part P4 + 1 parts MIX Diluent	6.25
P6	1 part P4 + 1 parts MIX Diluent	3.13
P7	1 part P4 + 1 parts MIX Diluent	1.56
P8	MIX Diluent	0.00

- **Biotinylated Albumin (1x):** Dilute Biotinylated Albumin with 4 ml MIX Diluent to produce a working solution. Allow it to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at  $< -20^{\circ}\text{C}$ .
- **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  $-20^{\circ}\text{C}$ .

## Assay Procedure

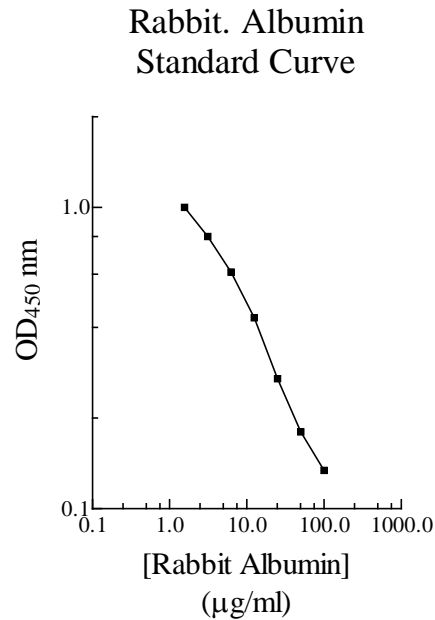
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature ( $20\text{-}30^{\circ}\text{C}$ ).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25  $\mu\text{l}$  of standard or sample per well, and immediately add 25  $\mu\text{l}$  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 two hours. Start the timer after the last sample addition.
- Wash five times with 200  $\mu\text{l}$  of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50  $\mu\text{l}$  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  $\mu\text{l}$  of Wash Buffer.
- Add 50  $\mu\text{l}$  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  $\mu\text{l}$  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.



## Precision, Sensitivity and Specificity

- The minimum detectable dose of Albumin is typically 100 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.5% respectively.

## Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
<b>1:1000</b>	100%	98%
<b>1:2000</b>	100%	103%
<b>1:4000</b>	102%	105%

Sample Dilution	Average Percentage of Expected Value
	Urine
<b>No Dilution</b>	96%
<b>1:2</b>	98%
<b>1:4</b>	105%

## Recovery

<b>Standard Added Value</b>	2 - 25 µg
<b>Recovery %</b>	85-110 %
<b>Average Recovery %</b>	97.5 %

## Cross-Reactivity

<b>Name</b>	<b>% Cross Reactivity</b>
Human Albumin	< 0.1
Mouse Albumin	< 0.1
Rat Albumin	< 0.1
Swine Albumin	< 0.1
Bovine Albumin	< 0.01

## References

- (1) Gekle M. (2004) *Annu Rev Physiol.*
- (2) Schindler C *et al.* (1999) *J Hepatol.* 31(6):1132
- (3) Hemmeler MH *et al.* (1997) *Nephrol Dial Transplant.* 12 Suppl 2:57-62
- (4) Sesnilo G *et al.* (2004) *Ann Intern Med.* 133(2):111-22
- (5) Wettstein R *et al.* (2004) *Shock.* 22(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 2.4

## Related Products

- EA2201-1 AssayMax Human Albumin ELISA Kit (Plasma and Serum samples)
- EA3201-1 AssayMax Human Albumin ELISA Kit (Urine and Cell Culture Supernatant samples)
- EMA2201-1 AssayMax Mouse Albumin ELISA Kit (Plasma and Serum samples)
- EMA3201-1 AssayMax Mouse Albumin ELISA Kit (Urine and Cell Culture Supernatant samples)
- ERA2201-1 AssayMax Rat Albumin ELISA Kit (Plasma and Serum samples)
- ERA3201-1 AssayMax Rat Albumin ELISA Kit (Urine and Cell Culture Supernatant samples)
- EPA3201-1 AssayMax Porcine Albumin ELISA Kit