Rabbit Albumin
ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

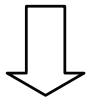
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

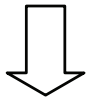
Thank you for choosing Assaypro.

Assay Summary

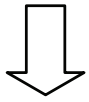
Add 25 µl of standard/sample
and 25 µl of biotinylated protein per well.
Incubate 2 hours.



Wash, then add 50 µl of SP per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 12 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

AssayMax Rabbit Albumin ELISA Kit

Catalog No. ETA2202-1
Sample Insert/Reference Only

Introduction

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

Principle 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, urine, and cell culture supernatant in less than 3 hours. A polyclonal chicken antibody specific for rabbit albumin has been pre-coated onto a 96-well microplate with removable strips. Albumin in standards and samples is competed with 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

- **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 acidic solution.

Reagents

- **Rabbit Albumin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal chicken antibody against rabbit albumin.
- **Sealing Tapes:** Each kit contains 3 precut, 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 (600 µg, lyophilized).
- **Biotinylated Rabbit 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 (80 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 components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store standard and biotinylated protein at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

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 3000 x *g* for 10 minutes. Dilute samples 1:10000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute samples 1:10000 into MIX Diluent and assay. The undiluted serum can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles**.**
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 into MIX Diluent and assay. The undiluted samples can be stored at -20°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.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 600 μg of Rabbit Albumin Standard with 3 ml of MIX Diluent to generate a solution of 200 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 stock (200 μg/ml) 1:4 with MIX Diluent to produce a 50, 12.5, 3.125, 0.781, and 0.195 μg/ml solutions. MIX Diluent serves as the zero standard (0 μg/ml). Any remaining solution should be frozen at -20°C and used within the next 30 days.

Standard Point	Dilution	[Rabbit Albumin] (μg/ml)
P1	1 part Standard (200 μg/ml) + 3 parts MIX Diluent	50.00
P2	1 part P1 + 3 parts MIX Diluent	12.50
P3	1 part P2 + 3 parts MIX Diluent	3.125
P4	1 part P3 + 3 parts MIX Diluent	0.781
P5	1 part P4 + 3 parts MIX Diluent	0.195
P6	MIX Diluent	0.000

- **Biotinylated Rabbit Albumin (1x):** Reconstitute Biotinylated Rabbit Albumin with 4 ml MIX Diluent to produce a working solution. Allow the biotin to sit for 10 minutes with gentle agitation and assay. Any remaining solution should be frozen at -20°C and used within the next 30 days.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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 (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 μl of Rabbit Albumin Standard or sample per well, and immediately add 25 μl of Biotinylated Rabbit Albumin to each well (on top of the standard or sample) and mix gently. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- 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.

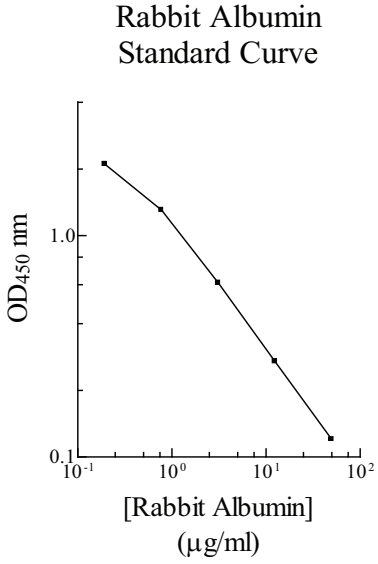
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. 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 generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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.

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 ~ 0.19 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.3% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:5000	104%	103%
1:10000	97%	98%
1:20000	95%	95%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
No Dilution	106%
1:2	98%
1:4	93%

Recovery

Standard Added Value	0.4 - 40 µg/ml
Recovery %	83-109%
Average Recovery %	98%

Cross-Reactivity

Name	% Cross Reactivity
Human	None
Mouse	None
Rat	None
Swine	None
Bovine	None
Rabbit	100%
Monkey	None
Canine	None

References

(1) Gekle M. (2004) *Annu 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*. 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 1.9

Related Products

- EA2201-1 AssayMax Human Albumin ELISA Kit (Plasma and Serum samples)
- EA3201-1 AssayMax Human Albumin ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EMA2201-1 AssayMax Mouse Albumin ELISA Kit (Plasma and Serum samples)
- EMA3201-1 AssayMax Mouse Albumin ELISA Kit (Urine and Cell Culture samples)
- ERA2201-1 AssayMax Rat Albumin ELISA Kit (Plasma and Serum samples)
- ERA3201-1 AssayMax Rat Albumin ELISA Kit (Urine and Cell Culture samples)
- EPA3201-1 AssayMax Swine Albumin ELISA Kit (Urine and Cell Culture samples)
- EPA2201-1 AssayMax Swine Albumin ELISA Kit (Plasma and Serum samples)