Swine Albumin
ELISA Kit

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

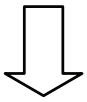
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

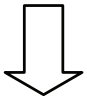
Thank you for choosing Assaypro.

Assay Summary

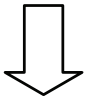
Add 50 µl of standard/sample per well.
Incubate 2 hours.



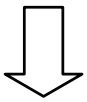
Wash, then add 50 µl of
biotinylated antibody per well.
Incubate 1 hour.



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



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 7 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 Swine Albumin ELISA Kit

Catalog No. EPA3201-1
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 Swine Albumin ELISA (Enzyme-Linked Immunosorbent Assay) kit employs a quantitative sandwich enzyme immunoassay technique that measures swine albumin in urine and cell culture supernatant in less than 4 hours. A polyclonal antibody specific for swine 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 swine 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

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, 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 and the biotinylated antibody 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

- **Swine Albumin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against swine albumin.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Swine Albumin Standard:** Swine albumin in a buffered protein base (3.2 μg, lyophilized).
- **Biotinylated Swine Albumin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against swine 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, 2 bottles).
- **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 and biotinylated antibody 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 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

- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:8000 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.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. 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 3.2 µg of Swine Albumin Standard with 4 ml of MIX Diluent to generate a stock solution of 800 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. The 800 ng/ml stock solution should be further diluted 1:8 with MIX Diluent to produce a 100 ng/ml standard solution. Prepare duplicate or triplicate standard points by serially diluting the standard solution (100 ng/ml) 1:2 with MIX Diluent to generate 50, 25, 12.5, 6.25, 3.125, and 1.563 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Swine Albumin] (ng/ml)
P1	1 part Standard (800 ng/ml) + 7 parts MIX Diluent	100.0
P2	1 part P1 + 1 part MIX Diluent	50.00
P3	1 part P2 + 1 part MIX Diluent	25.00
P4	1 part P3 + 1 part MIX Diluent	12.50
P5	1 part P4 + 1 part MIX Diluent	6.250
P6	1 part P5 + 1 part MIX Diluent	3.125
P7	1 part P6 + 1 part MIX Diluent	1.563
P8	MIX Diluent	0.000

- **Biotinylated Swine 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):** 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

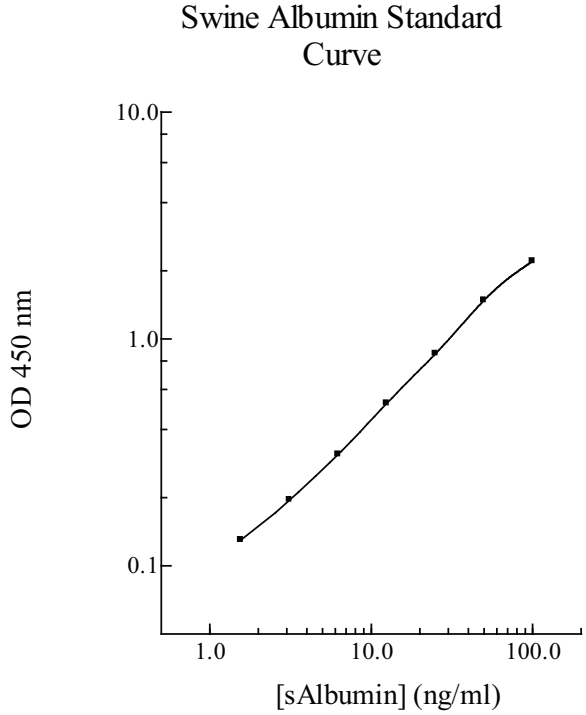
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Swine Albumin Standard or sample per well. 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 Biotinylated Swine Albumin Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- 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 7 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 swine albumin is typically ~ 1.5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.5 % respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:4000	93%
1:8000	99%
1:16000	104%

Recovery

Standard Added Value	1.5 - 25 ng/ml
Recovery %	89 - 108%
Average Recovery %	98%

Cross Reactivity

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

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

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 2.4

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)
- ERA3201-1 AssayMax Rat Albumin ELISA Kit (Urine and Cell Culture samples)
- ERA2201-1 AssayMax Rat Albumin ELISA Kit (Plasma and Serum samples)
- ETA2202-1 AssayMax Rabbit Albumin ELISA Kit (Plasma, Serum, Urine, and Cell Culture samples)
- EPA2201-1 AssayMax Swine Albumin ELISA Kit (Plasma and Serum samples)