RAT SERUM ALBUMIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF RAT ALBUMIN CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	RAT SERUM ALBUMIN ELISA
Catalog No.	SK00383-02
Lot No.	
Formulation	96 T
Standard Range	0.024-100 µg/mL
Sensitivity	12 ng/mL
Sample Volume	100 µl of diluted sample
Dilution Factor	2500 (**Optimal dilutions should be determined by each laboratory for each application**)
Sample Type	Serum and EDTA Plasma
Specificity	Rat Albumin
Intra-assay Precision	4-8%
Inter-assay Precision	8-12%
Storage	2°C – 8°C

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Rat albumin immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure rat albumin in serum and EDTA plasma. It has been shown to accurately quantify rat albumin. Results obtained with naturally occurring albumin samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for rat albumin.

PRINCIPLE OF THE ASSAY

The Rat Albumin ELISA kit is based on the binding of rat albumin in samples to two antibodies. One has been pre-coated onto a microplate, and the other is biotinylated. Standards and samples are pipetted into the wells and any albumin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for albumin is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of albumin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Rat Albumin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against rat albumin	**383-02-01**	**1 plate**
Rat Albumin Standard – 400 µg/vial of rat albumin for calibration in a buffered protein base with preservative; lyophilized.	**383-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of a biotinylated antibody against rat albumin with preservative; lyophilized.	**383-02-03**	**1 vial**
Positive Control – one vial of rat albumin, lyophilized.	**383-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugated to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60mL of buffered protein based solution with preservative.	**DB18**	**2 bottles**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive

Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution **(protect from light)** and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may need a 2500-fold dilution. A suggested 2500-fold dilution is 10 µL sample + 90 µL Dilution Buffer. Following 10 µL of 10x-diluted sample + 90 µL Dilution Buffer. Finally, 10 µL of 100x-diluted sample + 240 µL Dilution Buffer. **Notice:** *Albumin concentrations vary greatly, so optimal dilutions should be determined by each laboratory for each application with a pretest.* **Use polypropylene test tubes.**

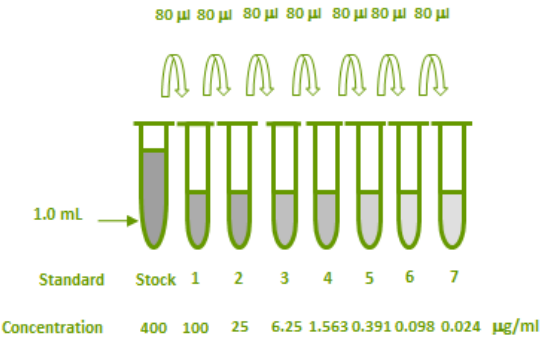
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Rat Albumin Standard - Refer to vial label for reconstitution volume. Reconstitute the Albumin Standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 400 µg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 240 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **100 µg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 µg/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 µl	400 µg/ml
# 1	80 µl of stock	240 µl	100 µg/ml
# 2	80 µl of 1	240 µl	25 µg/ml
# 3	80 µl of 2	240 µl	6.25 µg/ml
# 4	80 µl of 3	240 µl	1.563 µg/ml
# 5	80 µl of 4	240 µl	0.391 µg/ml
# 6	80 µl of 5	240 µl	0.098 µg/ml
# 7	80 µl of 6	240 µl	0.024 µg/ml



Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of

10-fold concentrated stock solution to prepare working solution.

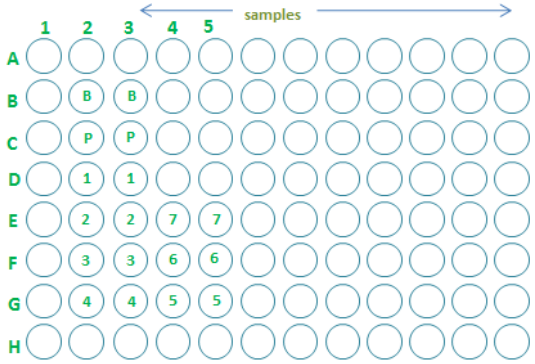
Streptavidin-HRP Conjugate - Pipette 11.94 mL of **HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make a positive control stock solution. To make positive control working solution, make a 5-fold dilution. A suggested 5-fold dilution is 50 µL of positive control stock solution + 200 µL Dilution Buffer. **Note:** Positive control working solution should be used immediately. Store positive control stock solution at -20°C ~ -70°C, it can be stored for a few days after reconstitution.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and close.
3. Add 100 µL of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 µL of **Standard** (in reverse order of serial dilution from E4, E5 to G4, G5 and G2, G3 to D2, D3), **sample**, or **positive control** (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 2-8 minutes on a micro-plate shaker at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the albumin concentrations versus the log of the O.D. and the best fit line can be determined by regression

analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified rat albumin.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of rat albumin was 12 ng/mL.

SPECIFICITY

This assay recognizes natural rat serum albumin. The factors listed below were prepared at 10,000 µg/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 10,000 µg/mL in a mid-range rat albumin control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY (%)
Rat serum albumin	100
Mouse serum albumin	0
Human serum albumin	0

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (µG/ML)	AVERAGE OD450 CORRECTED
Blank	0 (0.089)
0.024	0.008
0.098	0.015
0.391	0.064
1.563	0.214
6.25	0.529
25	1.035
100	1.511

- Lot No.:
- Positive Control:

LINEARITY

To assess the linearity of the assay, pooled research rat serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (µG/ML)	FINAL (MG/ML)	RECOVERY (%)
2500x	12.847	32.1175	100
25,000x	1.640	41.0	128

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of **standard, samples, positive control** to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Detection Antibody working solution** to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Streptavidin-HRP conjugate working solution** to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Substrate Solution** to each well. Incubate 2-8 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min.