

Human Albumin ELISA Quantitation Kit



Catalog No. **Size**
E80-129 1000 wells

Conditions

Storage: 2 – 8° C

Range of detection: 400 – 6.25 ng/ml

Shelf life: 1 year from date of receipt

Kit Materials

1. Coating Antibody

Goat anti-Human Albumin-affinity purified
Concentration: 1 mg/ml
Catalog No: A80-129A
Amount: 1 ml
Working Dilution: 1/100

2. Calibrator

Human Reference Serum
Catalog No: RS10-110
Amount: 0.1 ml
Working range: 400 – 6.25 ng/ml

3. HRP Detection Antibody

Goat anti-Human Albumin-HRP conjugate
Concentration: 1 mg/ml
Catalog No: A80-129P
Amount: 0.1 ml
Working Dilution: 1:10,000 – 1:200,000

Buffers, Substrate, and Plates not included

Materials Used in Our Assay

TMB/H₂O₂, 50-76-00, Kirkegaard and Perry, Gaithersburg, MD.
Nunc C bottom Immunoplate 96 well, 446612.
1% Bovine Serum Albumin in Tris Buffered Saline, T-6789, Sigma Chemical.

Application

The Human Albumin ELISA is used to quantitatively measure levels of Human Albumin in serum or other biological samples.

Production Procedures

By immunoelectrophoresis and ELISA the antibodies in this kit react specifically with Human Albumin.

Cross-reactivity with albumin from other species has not been tested.

Notes

The kit performance has been optimized for the stated protocol using the materials listed and standard dilutions from 400 to 6.25 ng/ml of Human Albumin. *For alternative assay conditions, the operator must determine appropriate dilutions of reagents.*

ELISA assay reactivity is sensitive to any variation in operator, pipetting and washing techniques, incubation time or temperature, composition of reagents, and kit age. Adjustments may be required to position the standard curve and/or samples in the desired detection range.

Country of Origin: USA

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

Product Specific References

1. D. B. Kuhns, E. L. Nelson, W. G. Alvord, J. I. Gallin, *J.Immunol.* 167, 2869-2878 (2001).

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Human Albumin Quantitative ELISA Protocol

A. Solutions and Reagents

Prepare the following buffers from the ELISA Starter Accessory Kit (E101) or your lab:

- A1. ■ Coating Buffer**
0.05 M Carbonate-Bicarbonate, pH 9.6
- A2. ■ Wash Solution**
50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0
- A3. ■ Blocking (Postcoat) Solution**
50 mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0
- A4. ■ Sample/Conjugate Diluent**
50 mM Tris, 0.14 M NaCl, 1% BSA, 0.05% Tween 20, pH 8.0
- A5. ■ Enzyme Substrate**
TMB (OPD or ABTS can be used)
- A6. ■ Stopping Solution**
2 M H₂SO₄ or other appropriate solution

B. Step-by-Step Method

(Perform all steps at room temperature.)

B1. ■ Coat with Capture Antibody

- ☐ Determine the number of single wells needed. Standards, samples, blanks and/or controls should be analyzed in duplicate. Insert the required number of microtiter well strips into a holder.
- ☐ Dilute 1 µl capture antibody (A80-129A) to 100 µl Coating Buffer for each well to be coated. (Example: for 32 wells dilute 32 µl to 3.2 ml)
- ☐ Incubate coated plate for 60 minutes.
- ☐ After incubation, aspirate the Capture Antibody solution from each well
- ☐ Wash each well with Wash Solution as follows:
 - ☐ Fill each well with Wash Solution
 - ☐ Remove Wash Solution by aspiration
 - ☐ Repeat for a total of 3 washes.

B2. ■ Blocking (Postcoat)

- ☐ Add 200 µl of Blocking (Postcoat) Solution to each well.
- ☐ Incubate 30 minutes.
- ☐ After incubation, remove the Blocking (Postcoat) Solution and wash each well three times as in Step B1.

B3. ■ Standards and Samples

- ☐ Dilute the standards in Sample Diluent according to the chart below:

Step	ng/ml	Calibrator RS10-110-3	Sample Diluent
0	10,000	5 µl	12.5 ml
1	400	.5 ml from step 0	12 ml
2	200	1 ml from step 1	1 ml
3	100	1 ml from step 2	1 ml
4	50	1 ml from step 3	1 ml
5	25	1 ml from step 4	1 ml
6	12.5	1 ml from step 5	1 ml
7	6.25	1 ml from step 6	1 ml

- ☐ Dilute the samples, based on the expected concentration of the analyte, to fall within the concentration range of the standards.
- ☐ Transfer 100 µl of standard or sample to assigned wells.
- ☐ Incubate plate 60 minutes.
- ☐ After incubation, remove samples and standards and wash each well 5 times as in Step B1.

B4. ■ HRP Detection Antibody

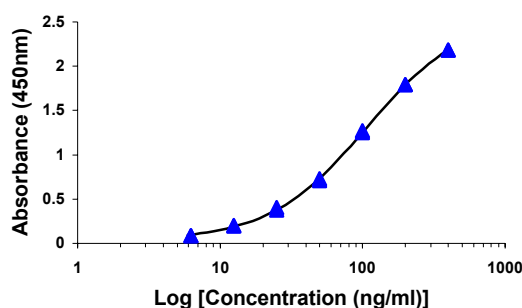
- ☐ Dilute the HRP Conjugate (A80-129P) in Conjugate Diluent. Recommended starting dilution is 1:70,000 with a range of 1:10,000 to 1:200,000. (Note: Adjustments in dilution may be needed depending on substrate used, incubation time, and age of kit.)
- ☐ Transfer 100 µl to each well.
- ☐ Incubate 60 minutes.
- ☐ After incubation, remove HRP Conjugate and wash each well 5 times as in Step B1.

B5. ■ Enzyme Substrate Reaction

- ☐ Prepare the substrate solution according to the manufacturer's recommendation.
- ☐ If using TMB from the ELISA Starter Accessory Kit, mix equal volumes of the two-substrate reagents.
- ☐ Transfer 100 µl of substrate solution to each well.
- ☐ Incubate plate 5 - 30 minutes.
- ☐ To stop the TMB reaction, apply 100 µl of 2 M H₂SO₄ to each well. If using another substrate, use the stop solution recommended by manufacturer.
- ☐ Using a microtiter plate reader, read the plate at the wavelength that is appropriate for the substrate used (450 nm for TMB)

C. Calculation of Results

- ☐ Average the duplicate readings from each standard, control, and sample.
- ☐ Subtract the zero reading from each averaged value above.
- ☐ Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. Other curve fits may also be used.
- ☐ A standard curve should be generated for each set of samples (See example below).



D. Troubleshooting

D1. ■ Problem: Low absorbance

- ☐ Incorrect dilutions or pipetting errors
- ☐ Improper incubation times
- ☐ Improper mixing of the TMB substrate. Each component is mixed in equal parts.
- ☐ Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
- ☐ Kit materials or reagents are contaminated or expired.
- ☐ Incorrect reagents used.

D2. ■ Problem: High Absorbance

- ☐ Cross contamination from other samples or positive control
- ☐ Incorrect dilutions or pipetting errors
- ☐ Improper washing
- ☐ Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
- ☐ Contaminated buffers or enzyme substrate
- ☐ Improper incubation times
- ☐ Kit materials or reagents are contaminated or expired.

D3. ■ Problem: Poor Duplicates

- ☐ Poor mixing of specimens
- ☐ Incorrect dilutions or pipetting errors
- ☐ Technical error
- ☐ Inconsistency in following ELISA protocol
- ☐ Inefficient washing

D4. ■ Problem: All wells are positive

- ☐ Contaminated buffers or enzyme substrate
- ☐ Incorrect dilutions or pipetting errors
- ☐ Kit materials or reagents are contaminated or expired.
- ☐ Inefficient washing

D5. ■ Problem: All wells are negative

- ☐ Procedure not followed correctly
- ☐ Contaminated buffers or enzyme substrate
- ☐ Contaminated Conjugate
- ☐ Kit materials or reagents are contaminated or expired.

E. Technical Hints

- ☐ When preparing coating buffer from the gel capsule, break the capsule apart and pour ingredients into water. Do not place gel capsule into water. The gelatin from the capsule interferes with the binding of the coating antibody to the plate.
- ☐ Capture antibody diluted with coating buffer should be added to wells immediately.
- ☐ Coated (covered) plates are stable overnight at 4°C.
- ☐ Check all buffers for contamination and expiration. When trouble shooting, it may be helpful to start with all new buffers. Make buffers in new or properly cleaned vessels.
- ☐ Sodium Azide should not be added to any of the buffers.
- ☐ Dilutions should be made shortly before application and immediately applied to appropriate wells.
- ☐ Wash buffer should be aspirated from wells. Pouring/Dumping wash buffer from wells may lead to cross contamination.
- ☐ Excess antibody/analyte should be wiped from pipettes tips when making dilutions.
- ☐ Incubation time of the Enzyme Substrate will depend on the substrate used and the intensity of the color change. The high standard should have an O.D. reading of about 2.0 and the O.D. reading of the low standard should be above background.
- ☐ Stop solution should be added to the plate in the same order as the Enzyme Substrate.

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MATERIAL SAFETY DATA SHEET

Contents Description

This product contains sheep or goat affinity purified antibody, sheep or goat HRP conjugated affinity purified antibody and reference serum.

Hazardous Ingredients

Affinity Purified Antibody - No known toxicity for this biological material.

Reference Serum - No known toxicity for this biological material. Serum or plasma from each donor has been tested and found negative for HIV antibody and HbSAg. HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

Horseradish Peroxidase (HRP)- No known toxicity for this plant material.

Bovine Serum Albumin (BSA) - No known toxicity for this biological material.

Thimerosal - May be fatal if inhaled, swallowed or absorbed through the skin. Can cause eye and skin irritation. Wash all affected areas with large volumes of water. If swallowed consult your physician immediately. If inhaled remove to fresh air. If necessary, give artificial respiration by mouth-to mouth.
LD50 oral rat - 75 mg/kg.

Sodium Azide (NaN_3) - AZIDE FORMS EXPLOSIVE CHEMICAL COMPOUNDS WITH LEAD AND COPPER PLUMBING. CARE MUST BE TAKEN TO WASH WASTE DOWN DRAINS WITH LARGE VOLUMES OF WATER.

LD50 oral mouse - 27 mg/kg.

Wash all affected areas with large volumes of water and if swallowed consult your physician immediately.

The above information is believed to be correct but does not purport to be all-inclusive and is intended to be used only as a guide. Bethyl Laboratories, Inc. shall not be liable or responsible in any way for use of either this information or the material supplied. Disposal of hazardous material may be subject to federal, state, or local laws or regulations.

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