

# **LSBio™ Human Alpha-2- Macroglobulin / A2M ELISA Kit**

**Catalog No. LS-F10409**

## **User Manual**

**Please Read the Manual Carefully  
Before Starting your Experiment**



For research use only. Not approved for use in humans or for clinical diagnosis.

**INTENDED USE**

This human alpha-2-macroglobulin (A2M) assay is for the quantitative determination of A2M in human plasma and other biological fluids. **For research use only.**

**BACKGROUND**

A2M is a circulating 720 kDa homotetramer expressed in the liver which captures a wide range of plasma proteinases including plasmin and thrombin. Each monomer contains an internal thiol ester, a transglutaminase reactive site, zinc and receptor binding sites, and a bait region which when cleaved induces a conformational change trapping the proteinase [1]. Serum A2M levels are decreased in acute pancreatitis [2] and increased in chronic liver disease and nephrotic syndrome [3].

**ASSAY PRINCIPLE**

Human A2M will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, polyclonal anti-human A2M primary antibody binds to the A2M. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. Following an additional washing step, TMB substrate is used for color development at 450nm. The amount of color development is directly proportional to the concentration of total A2M in the sample.

**REAGENTS PROVIDED**

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human A2M antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human A2M standard:** 1 vial lyophilized standard
- **Anti-human A2M primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-chicken horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

**STORAGE AND STABILITY**

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

**OTHER REAGENTS AND SUPPLIES REQUIRED**

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H<sub>2</sub>SO<sub>4</sub> or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

**PRECAUTIONS**

- **FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.**
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

## PREPARATION OF REAGENTS

- TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- Blocking buffer (BB):** 3% BSA (w/v) in TBS
- 1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

## SAMPLE COLLECTION

Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

## ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

### Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of human A2M standard:

A2M concentration (ng/ml)	Dilutions
250	750 $\mu\text{l}$ (BB) + 250 $\mu\text{l}$ (1000ng/ml)
125	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (250ng/ml)
50	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (125ng/ml)
25	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (50ng/ml)
10	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (25ng/ml)
5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (10ng/ml)
2.5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (5ng/ml)
1	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (2.5ng/ml)
0	500 $\mu\text{l}$ (BB) Zero point to determine background

**NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.**

### Standard and Unknown Addition

Remove microtiter plate from bag and add 100 $\mu\text{l}$  A2M standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures A2M in the 1-250 ng/ml range. If the unknown is thought to have high A2M levels, dilutions may be made in blocking buffer. A 1:100,000 dilution for normal human plasma is suggested for best results.

### Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 3 $\mu\text{l}$  of conjugated secondary antibody in 10ml of blocking buffer and add 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Substrate Incubation

Add 100 $\mu\text{l}$  TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 $\mu\text{l}$  of 1N  $\text{H}_2\text{SO}_4$  or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

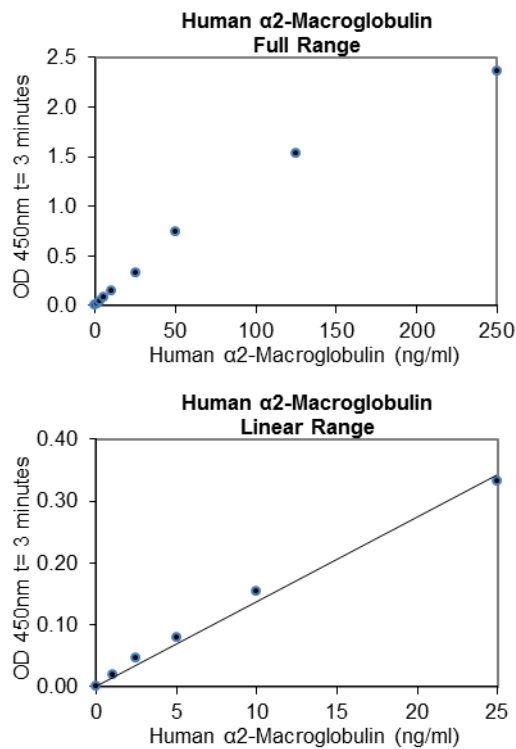
### Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ( $A_{450}$ ).

## Calculation of Results

Plot A<sub>450</sub> against the amount of A2M in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of A2M in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



## EXPECTED VALUES

The concentration of A2M in pooled donor plasma from normal individuals was found to be 1.2 mg/ml [4].

## PERFORMANCE CHARACTERISTICS

**Sensitivity:** The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD<sub>450</sub>: 0.163-0.176) and calculating the corresponding concentration. The MDD was 0.20 ng/ml.

**Intra-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Inter-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Recovery:** These studies are currently in progress. Please contact us for more information.

**Linearity:** To assess the linearity of the assay, pooled citrated human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	112	86	97	89
Range	99-121%	84-89%	91-101%	79-97%

**Specificity:** These studies are currently in progress. Please contact us for more information.

**Sample Values:** Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (mg/mL)
Citrate Plasma	1:30,000	1.31
	1:60,000	1.48
	1:120,000	1.14
	1:240,000	1.27
	1:480,000	1.17

## DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

## REFERENCES

1. Borth W: FASEB J. 1992, 6:3345-53.
2. McMahon MJ, *et al.*: Am J Surg. 1984, 147(1):164-70.
3. Housley J: J Clin Path. 1968, 21:27-31.
4. Coan MH and Roberts RC: Biol Chem. 1989, 370:673-6.

### Example of ELISA Plate Layout

96 Well Plate: 18 Standard wells, 78 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	125 ng/ml	250 ng/ml			
B	0	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	125 ng/ml	250 ng/ml			
C												
D												
E												
F												
G												
H												





**Important Note:** During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

**Warning:** This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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