

Human Alpha-2-Macroglobulin ELISA Kit

Catalog No: CKH349A
Catalog No: CKH349B

Size: 1 x 96 wells
Size: 5 x 96 wells

Intended use:

This Human Alpha-2-Macroglobulin (A2M) ELISA assay is for the quantitative determination of A2M in human plasma and other biological fluids. The kit has been formulated **For Research Use Only**.

Background:

A2M is a circulating 720 kDa homotetramer expressed in the liver that 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. Serum A2M levels are decreased in acute pancreatitis and increased in chronic liver disease and nephrotic syndrome.

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 for 1 x 96 Wells:

Items	Quantity
A: Microtiter plate coated, blocked and dried with Anti-Human Alpha-2 Macroglobulin	1 96-well coated microtiter strip plate (with 8 x12-well removable strips)
B: Wash Buffer Concentrate (10x)	1 bottle of 50 ml
C: Human A2M Standard	1 vial (lyophilized)
D: Anti-Human A2M Primary pAb	1 vial (lyophilized)
E: HRP Conjugated anti-Chicken Secondary Ab	1 vial concentrated solution
F: TMB Substrate Solution*	1 bottle of 10 ml

*Hazard Information:

Avoid skin and eye contact when using TMB substrate solution as it may be irritating to eyes, skin and respiratory system. Wear safety goggles and gloves.

Storage and Stability:

All kit components must be stored at 4°C upon arrival. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary antibody may be stored at -80°C for later use. **DO NOT freeze/thaw the standards and primary antibody more than once.** All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.



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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
- 1 N H₂SO₄ or 1 N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

Precautions:

- **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 substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- 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:** 0.1 M Tris + 0.15 M NaCl, pH 7.4.
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50 ml of 10X wash buffer concentrate with 450 ml of deionized water.

Sample Collection:

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

Assay Procedure:

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

1. Preparation of Standard

Reconstitute standard by adding 1 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000 ng/ml standard solution.



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Dilution table for preparation of Human A2M standard:

Human A2M Concentration (ng/ml)	Dilutions
250	750 µl (BB) + 250 µl (1,000 ng/ml)
125	500 µl (BB) + 500 µl (250 ng/ml)
50	600 µl (BB) + 400 µl (125 ng/ml)
25	500 µl (BB) + 500 µl (50 ng/ml)
10	600 µl (BB) + 400 µl (25 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2.5	500 µl (BB) + 500 µl (5 ng/ml)
1	600 µl (BB) + 400 µl (2.5 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

2. Standard and Unknown Addition

Remove microtiter plate from bag and add 100 µl A2M standards (in duplicate) and unknowns to wells. Carefully record the position of the standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

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

3. Primary Antibody Addition

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

4. Secondary Antibody Addition

Dilute 1 µl of conjugated secondary antibody in 10 ml of blocking buffer and add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

5. Substrate Incubation

Add 100 µl 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 µl of 1 N H₂SO₄ 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.

6. Measurement

Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. For best results read plate immediately. Subtract zero point from all standards and unknowns to determine corrected absorbance (A₄₅₀).



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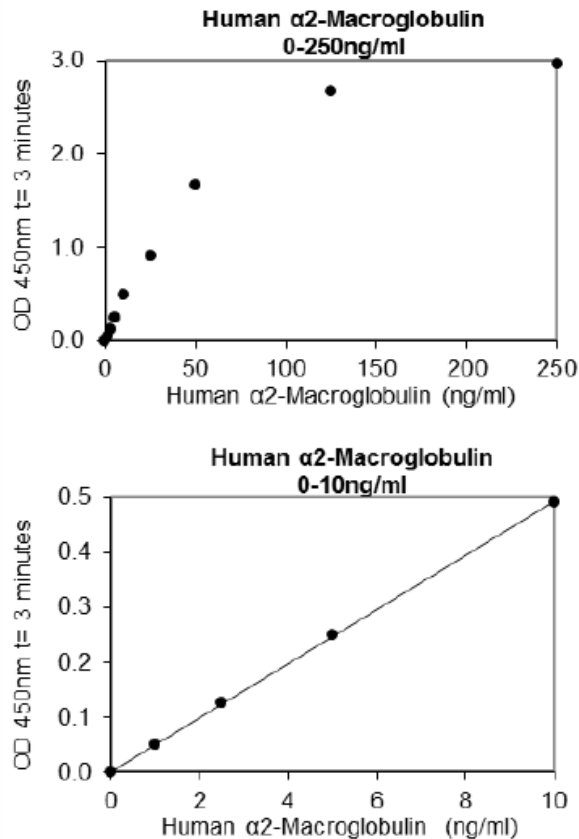
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7. Calculation of Results

Plot the A_{450} values against the amount of A2M in the standards. Fit a straight line through the points 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 human individuals was found to be 1.2 mg/ml.

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_{450} : 0.163-0.176) and calculating the corresponding concentration. The MDD was 0.20 ng/ml.



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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%

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

**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: This is a generic data sheet and may be subject to change. Please see the package insert shipped with your product for current data.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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