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User's Manual

AMBP (Human) ELISA Kit

REF **DEIA3130**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

AMBP (Human) ELISA Kit is a sandwich enzyme immunoassay for the quantitative measurement of human alpha1-microglobulin.

General Description

Alpha-1-microglobulin (A1M), also called protein HC, is a tubular plasma and tissue protein that belongs to the lipocalin transport protein superfamily for small hydrophobic molecules. It contains 184 amino acids and has a molecular weight of 26 kDa. Mature A1M and bikunin (urinary trypsin inhibitor) result from a common precursor. A1M is found in blood, both in free form and complex-bound to immunoglobulin A (IgA). It is involved in inflammatory and detoxification processes caused by immune system activation and extracellular heme catabolism. While increased excretion is detected in urine or serum shortly after tubular injury, A1M may predict acute kidney injury and the need for renal replacement therapy. Urinary A1M is useful for the early detection of nephropathy in type 2 diabetic subjects.

Principles of Testing

The AMBP (Human) ELISA Kit is designed for detection of alpha-1-microglobulin in human plasma, serum, milk, urine, saliva, CSF, cell culture, and cell lysate samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human alpha-1-microglobulin in approximately 4 hours. A polyclonal antibody specific for human alpha-1-microglobulin has been pre-coated onto a 96-well microplate with removable strips. Alpha-1-microglobulin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human alpha-1-microglobulin, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Human alpha-1-Microglobulin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human alpha-1-microglobulin. 96 (8×12) wells
2. Sealing Tapes: Pressure Sensitive sealing tapes that can be cut to fit the format of the individual assay. 3 slices
3. Human alpha-1-Microglobulin Standard: Human alpha1-microglobulin in a buffered protein base (lyophilized). 14 ng
4. Biotinylated Human alpha-1-Microglobulin Antibody (50×): A 50-fold concentrated biotinylated polyclonal antibody against human alpha-1-microglobulin. 120 µL
5. EIA Diluent Concentrate (10×): A 10-fold concentrated buffered protein base. 30 mL
6. Wash Buffer Concentrate (20×): A 20-fold concentrated buffered surfactant. 30 mL × 2
7. SP Conjugate (100×): A 100-fold concentrate. 80 µL
8. Chromogen Substrate (1×): A stabilized peroxidase chromogen substrate tetramethylbenzidine. 7 mL

9. Stop Solution (1×): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction. 11 mL

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
3. Deionized or distilled reagent grade water.

Storage

1. Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
2. Store SP Conjugate and Biotinylated Antibody at -20°C.
3. Store Microplate, Diluent Concentrate (10×), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
4. 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.
5. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent

Specimen Collection And Preparation

1. Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 × g for 10 minutes and collect plasma. A 10000-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
2. Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 × g for 10 minutes and remove serum. A 10000-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
3. Milk: Collect milk using sample tube. Centrifuge samples at 800 × g for 10 minutes. A 100-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
4. Urine: Collect urine using sample pot. Centrifuge samples at 800 × g for 10 minutes. A 500-fold sample dilution is suggested into EIA Diluent or within the range of 200× –2000×; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
5. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 × g for 10 minutes. A 4-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
6. CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 × g for 10 minutes. A

100-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

7. Cell Culture Supernatant: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
8. Cell Lysate: Rinse cell with cold PBS and then scrape the cell into a tube with 5 mL of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH 8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1×10^6 cells, add approximately 100 µL of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to dilution guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A) 4 µL sample : 396 µL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL.	A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A) 4 µL sample : 396 µL buffer (100x) B) 24 µL of A : 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL.	A) 4 µL sample: 396 µL buffer (100x) B) 4 µL of A : 396 buffer (100x) C) 24 µL of B : 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL.

Reagent Preparation

1. Freshly dilute all reagents and bring all reagents to room temperature before use.
2. EIA Diluent Concentrate (10×): Dilute the EIA Diluent Concentrate 10-fold with reagent grade water to produce a 1× solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1× solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
3. Human alpha-1-Microglobulin Standard: Reconstitute the Human alpha1-Microglobulin Standard (14 ng) with 0.7 mL of EIA Diluent to generate a 20 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (20 ng/mL) 2-fold with equal volume of EIA Diluent to produce 10, 5, 2.5, 1.25, 0.625, and 0.313 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and used within 30 days.

Standard Point	Dilution	[A1M] (ng/mL)
P1	1 part Standard (20 ng/mL)	20.00
P2	1 part P1 + 1 part EIA Diluent	10.00
P3	1 part P2 + 1 part EIA Diluent	5.000
P4	1 part P3 + 1 part EIA Diluent	2.500
P5	1 part P4 + 1 part EIA Diluent	1.250
P6	1 part P5 + 1 part EIA Diluent	0.625
P7	1 part P6 + 1 part EIA Diluent	0.313
P8	EIA Diluent	0.000

4. Biotinylated Human alpha-1-Microglobulin Antibody (50×): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with EIA Diluent to produce a 1× solution. The undiluted antibody should be stored at -20°C.
5. Wash Buffer Concentrate (20×): Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1× solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1× solution gently until the crystals have completely dissolved.
6. SP Conjugate (100×): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with EIA Diluent to produce a 1× solution. The undiluted conjugate should be stored at -20°C.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Human alpha-1-Microglobulin Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µL of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µL of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of Biotinylated Human alpha-1-Microglobulin Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. 8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 8 minutes or until the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to

ensure thorough mixing. Break any bubbles that may have formed.

11. 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.

Calculation

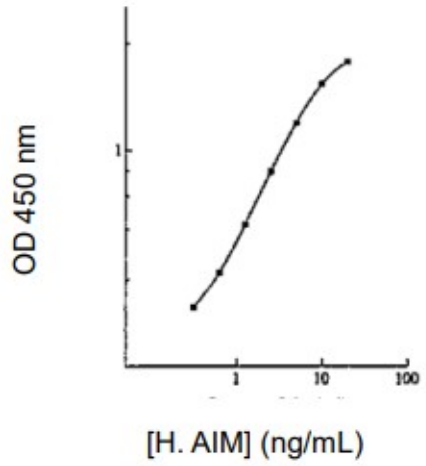
1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical Standard Curve

The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be cause by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	20.00	2.329 2.419	2.374
P2	10.00	1.942 1.874	1.908
P3	5.000	1.287 1.333	1.310
P4	2.500	0.789 0.839	0.814
P5	1.250	0.478 0.494	0.486
P6	0.625	0.290 0.314	0.302
P7	0.313	0.207 0.223	0.215
P8	0.000	0.111 0.107	0.109
Sample: Pooled Normal Sodium Citrate Plasma (10000x)		1.190 1.276	1.233

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

Normal human alpha-1-microglobulin plasma levels range from 31.5 - 58.5 µg/mL.

Plasma and serum samples from healthy adults were tested (n=40). On average, human alpha-1-microglobulin level was 44.3 µg/mL.

Precision

Intra-assay precision was determined by testing three plasma samples twenty times in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	5.9%	5.8%	4.4%	9.7%	10.4%	9.5%
Average CV (%)	5.4%			9.9%		

Detection Limit

The minimum detectable dose of human alpha-1-microglobulin as calculated by 2SD from the mean of a zero standard was established to be 0.16 ng/mL.

Specificity

Species	Cross-Reactivity (%)
Canine	None
Bovine	None
Monkey	80%
Mouse	None
Rat	None
Swine	None
Rabbit	None

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

Linearity

Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
5000x	95%	93%
10000x	101%	98%
20000x	106%	105%

Recovery

Recovery was determined by spiking two plasma samples with different alpha-1-microglobulin concentrations.

Sample	Unspiked Sample (ng/mL)	Spiked Sample (ng/mL)	Expected	Observed	Recovery (%)
1	5.0	15.0	20.00	19.9	100%
		5.0	10.00	9.5	95%
		2.0	7.0	6.9	99%
2	2.0	15.0	17.0	16.8	99%
		5.0	7.0	6.4	91%
		2.0	4.0	3.6	90%
Average Recovery (%)					96%

Precautions

1. This product is for Research Use Only and is not intended for use in diagnostic procedures.
2. Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
3. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
4. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
5. The Stop Solution is an acidic solution.
6. The kit should not be used beyond the expiration date.