



**User's Manual**

# Human $\alpha$ 1-Microglobulin ELISA kit

REF

DEIA-BJ216



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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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**Creative Diagnostics**

 **Address:** 45-1 Ramsey Road, Shirley, NY 11967, USA

 **Tel:** 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  **Fax:** 1-631-938-8221

 **Email:** [info@creative-diagnostics.com](mailto:info@creative-diagnostics.com)  **Web:** [www.creative-diagnostics.com](http://www.creative-diagnostics.com)

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## PRODUCT INFORMATION

### Intended Use

The A1M test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring A1M in Human biological samples. If the ELISA is to be used outside the intended use, the user may need to optimize for said use.

### Principles of Testing

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Alpha 1-Microglobulin (A1M) present in samples reacts with the anti-A1M antibodies, which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-A1M antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound A1M. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of A1M in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of A1M in the test sample. The quantity of A1M in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

### Reagents And Materials Provided

**The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per this kit protocol insert.**

- 1. ELISA Micro Plate, antibody coated:** One plate of 12 removable 8 well strips, antibody coated. 4-8°C, In sealed foil bag with desiccant. The plate strips are stable until the expiration date if stored correctly.
- 2. Enzyme Conjugated Detection Antibody:** One vial of 150 $\mu$ L of 100x Horseradish Peroxidase Conjugated antibody in a stabilizing buffer. 4-8°C in the dark. The working conjugate solution should be diluted immediately prior to use. The 100x conjugate is stable until the expiration date.
- 3. Calibrator:** One vial of lyophilized calibrator. 4-8°C for lyophilized calibrator. Aliquoted and frozen if re-constituted. Avoid multiple freezethaw cycles. The working standard solutions should be prepared immediately prior to use.
- 4. Diluent Concentrate:** One 50 mL bottle of 5x diluent buffer. 4-8°C for both 1x working solution and 5x diluent concentrate. The 1x working solution is stable for at least one week from the date of preparation. The 5x concentrate is stable until the expiration date
- 5. Wash Solution Concentrate:** One 50 mL bottle of 20x wash solution. 4-8°C for both 1x working solution and 20x diluent concentrate. The 1x working solution is stable for at least one week from the date of preparation. The 20x concentrate is stable until the expiration date.
- 6. Chromogen-Substrate Solution:** One bottle of 12 mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3. 4-8°C in the dark. Protect from light. The Substrate Solution is stable until the expiration date.
- 7. STOP Solution: WARNING: Avoid Contact with Skin.** 4-8°C. The Stop Solution is stable until the

expiration date.

## Materials Required But Not Supplied

1. Precision pipettes (2  $\mu$ L to 100  $\mu$ L) for making and dispensing dilutions
2. Test tubes
3. Squirt bottle or Microtitre washer/aspirator
4. Distilled or Deionized H<sub>2</sub>O
5. Microtitre Plate reader
6. Assorted glassware for the preparation of reagents and buffer solutions
7. Centrifuge for sample collection
8. Anticoagulant for plasma collection
9. Timer

## Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date

## Specimen Collection And Preparation

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.

If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

**1. Serum samples:** Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

**2. Plasma samples:** Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

**3. Known interfering substances:** Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

## DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

**1. Serum samples:** Recommended starting dilution is 1/300. To prepare a 1/300 dilution of a sample,

transfer 2  $\mu$ L of sample to 598  $\mu$ L of 1 $\times$  diluent. This gives you a 1/300 dilution. Mix thoroughly.

**2. Plasma samples:** Recommended starting dilution is 1/300. To prepare a 1/300 dilution of a sample, transfer 2  $\mu$ L of sample to 598  $\mu$ L of 1 $\times$  diluent. This gives you a 1/300 dilution. Mix thoroughly.

## Reagent Preparation

1. Bring all reagents to room temperature (16-25°C) before use.
2. Diluent Concentrate: The Diluent Solution supplied is a 5 $\times$  Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH<sub>2</sub>O).
3. Wash Solution Concentrate: The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH<sub>2</sub>O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.
4. Enzyme-Antibody Conjugate: Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10  $\mu$ L Enzyme-Antibody Conjugate to 990  $\mu$ L of 1 $\times$  Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.
5. Pre-coated ELISA Micro Plate: Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
6. Human A1M Calibrator: Prepare according to the Certificate of Analysis.

## Assay Procedure

1. All samples and standards should be assayed in duplicates.
2. Pipette 100  $\mu$ L of
  - Standard 0 (0.0 ng/mL) in duplicate
  - Standard 1 (12.5 ng/mL) in duplicate
  - Standard 2 (25 ng/mL) in duplicate
  - Standard 3 (50 ng/mL) in duplicate
  - Standard 4 (100 ng/mL) in duplicate
  - Standard 5 (200 ng/mL) in duplicate
  - Standard 6 (400 ng/mL) in duplicate
3. Pipette 100  $\mu$ L of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate at room temperature for sixty (60  $\pm$  2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

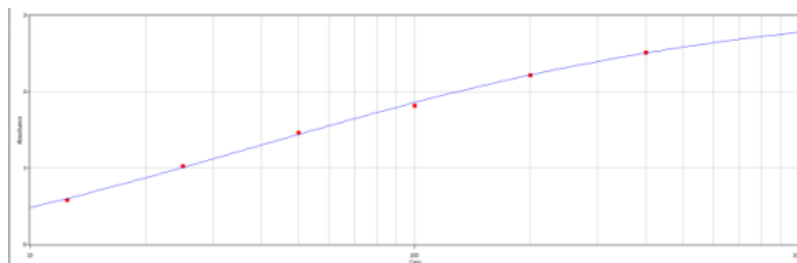
7. Pipette 100  $\mu$ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for sixty ( $60 \pm 2$ ) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100  $\mu$ L of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100  $\mu$ L of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

## Calculation

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the A1M concentration in original samples.

## Typical Standard Curve

Sample Standard Curve is for demonstration only. Do not use for results calculations. A standard curve should be generated each time the test is performed.



Standard #	Conc ng/mL	Replicates	Back Calc	% Recovery
1	12.5	0.582	12.094	96.75
2	25	1.025	25.74	102.96
3	50	1.462	51.846	103.69
4	100	1.821	94.27	94.27
5	200	2.22	201.656	100.83
6	400	2.511	406.736	101.68

## Precision

Intra-Assay Precision	Average %CV = 3.438 %	N = 24
Inter-Assay Precision	Average %CV = 5.746 %	N = 24

## Sensitivity

The lowest detection limit (LDL) is 2.481ng/mL.

The lowest detection limit is determined by adding three standard deviations to the mean optical density value of twenty-four Standard zero replicates and calculating the corresponding concentration.

## Limitations

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC PURPOSES. IN VITRO USE ONLY.

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.