DRG

DRG® Micro-Albumin ELISA (EIA-2361)

Revised 11 Nov. 2010 rm (Vers. 2.1) **USA: RUO**

NAME AND INTENDED USE

This Micro-Albumin Test is a solid phase enzyme-linked immunosorbant assay (ELISA). This test provides measurement of Micro-Albumin in human urine.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

This Micro-Albumin Quantitative test system is a solid phase enzyme-linked immunosorbant assay (ELISA). The wells are coated with specific albumin. The samples, standards and controls are incubated in the wells with anti-albumin enzyme conjugate. Enzyme conjugate and albumin in the urine sample compete binding with albumin antigens in the well. Unbound enzyme conjugate is washed off with water. The amount of bound peroxidase is inverted proportional to the concentration of the albumin present in the samples, standards and controls. Upon addition of the substrate and chromogen a color is developed after a short incubation period. The enzyme reaction is stopped and the intensity of the color measured with microwell reader at 450 nm. When high levels of albumin are present in sample's urine, less enzyme conjugate is bound, hence less color development is observed.

WARNINGS AND PRECAUTIONS

1. This kit is intended for Research Use Only. Not for use in diagnostic procedures.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.
3. References contains human serum should be treated as potentially infectious. All human based products should be used appropriate precautions.

MATERIALS PROVIDED

1. Microwell Strips (96 wells): Specific Micro-Albumin coated wells. 8x12 strips.
2. Enzyme Conjugate (11mL): Anti-Micro-Albumin Antibodies conjugated to horseradish peroxidase.
3. Sample Diluent (11 mL) or zero standard. Phosphate buffered saline with stabilizer.
4. Reference Standard Set (0.5 mL/each): Human MICRO-ALBUMIN. References: 2.5, 5, 25, 50, 100 ug/mL. Human Albumin Standard in the phosphate buffered saline. The Standards are calibrated to 0, 2.5, 5, 25, 50 and 100 ug/mL.
5. Control (0.5 ml) values as indicated on vial.
6. TMB Solution (11 ml): Buffer Solution containing hydrogen peroxide and TMB.
7. Washing Buffer Concentrate (20X) (50 mL): Prepare working washing solution by adding 50 mL washing buffer concentrate into 990 mL distilled water.
8. Stop solution (11 mL): 2N HCl
9. Well holder for securing individual well.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microwell reader with wavelength at 450 nm.
2. Pipetor with tips for measuring 25 uL, 100 uL.

3. 1 L washing bottle.

STORAGE AND STABILITY

1. Store the kits at 2-8°C and keep microwells in a dry bag with desiccants.
2. Reagents are stable until expiration of the kit. TMB Solution should be colorless. If the solutions turn blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING

Collect a single void, timed fractional, overnight or 24-hour sample without preservative-recording the time, duration and total volume of the collection. Mix well, withdraw a small portion, and clear by centrifugation or filtration. Prior to assay, allow the samples to come to room temperature. Do not thaw samples by applying heat. The following precautions should be observed when testing for urinary albumin:

1. Urinary albumin excretion is increased by physiological factors such as the erect posture, exercise and acute diuresis. Urine samples should not be collected undue exertion nor after an acute fluid load. Reference ranges must specify the type of urine collection.
2. The possibility of contamination with menstrual or seminal fluid or due to urinary tract infection should be borne in mind.
3. The urine specimen are kept at room temperature for a few minutes or stored at 4-8oC for a few hours or three days at most before assay. Frozen samples with Tween 20 (1mL/L) can be stored up to six months.

PREPARATION FOR ASSAY

1. Bring all reagents and samples to room temperature (24±3oC) and mix gently before beginning the test
2. Have all reagents and samples ready before the start of the assay. Once the test has begun, it must be performed without any interruption to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Dispense 10 uL of samples, controls and Standards into the assigned wells.
3. Dispense 100 uL of Enzyme conjugate into each well and mix for 5 seconds.
4. Incubate for 30 minutes at Room Temp.
5. Remove incubation mixture and rinse the wells five times with Washing Buffer (300 µL/well/each rinse).
6. Dispense 100 uL of TMB Solution into each well including the blank well.
7. Incubate for 15 minutes at R.T.
8. Stop reaction by adding 50 uL of Stop solution to each well and read O.D. at 450 nm with a microwell reader.

PROCEDURAL NOTE

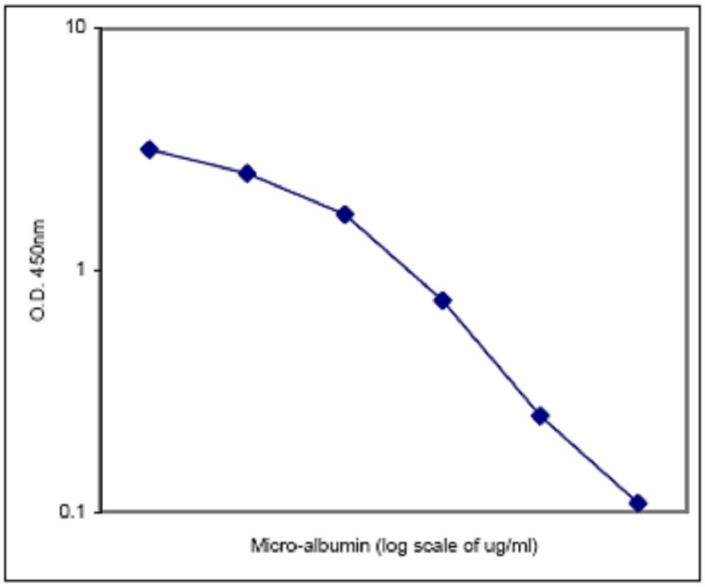
1. Wash the microwells and remove washing buffer thoroughly to get the Best results.
2. Pipet all reagents and samples into bottom of the well. Vortex-mixing of shaking is not required.
3. Absorbance is a function of the time and temperature of incubations. It is recommended to have reagents, samples and needed wells ready. Ensure the equal elapsed time for each pipetting without interruption.

4. For the same reason the size of the of the assay run should be limited. It is suggested to run no more than 20 samples with a set of Reference Standards in duplicate.
5. If a serum specimen contains greater than 100 ug/mL or Micro-albumin, the sample must be diluted with sample diluent and reassayed as described in the assay procedure.

CALCULATION OF RESULTS

1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on the graph paper.
2. Obtain the values of samples by reference to the Standard curve. (Following data is for demonstration purpose only).

Well No.	Description (ug/mL)	Absorbance (450 nm)	Micro-Albumin (ug/mL)
A1	0	3.157	
B1	2.5	2.505	
C1	5	2.067	
D1	25	0.458	
E1	50	0.250	
F1	100	0.109	
G1	Sample 1	1.222	9.7
H1	Sample 2	0.696	16.9
A2	Sample 3	0.555	20.8
B2	Sample 4	0.212	56.9
C2	Sample 5	0.175	76.0



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