پادتن علم
PADTAN ELM

THE PADTAN ELM EIA KIT FOR 96 TESTS

MicroAlbumin EIA Kit

Intended use:
Quantitative determination of Albumin levels in human urine.

For *in vitro* diagnostic use.

I.Introduction

Albumin , with a relative molecular weight of 66 kDa, is the major protein in human plasma, In the urine , under normal physiological conditions, albumin is present at very low concentrations.
Presence of increased quantities of protein in the urine(Proteinuria>200 µg/min) is an important predictor of progressive kidney damage. Micro-Albuminuria is preceded by a period of minor increased urinary albumin extraction (30 to 300 mg/day) not detected by routine methods.This period, designated as microalbuminuria is a marker of both renal and cardiovascular disease. If microalbuminuria remains undetected, it leads to subsequent development of diabetic nephropathy and irreversible kidney failure. Thus, in order to detect minimal renal impairment and to control its progression a sensitive method of measuring minor increases in urinary albumin is of great importance .
Although testing for microalbuminuria is primarily indicated in diabetes mellitus(1-5) , it has also been used in studies of hypertension, pregnancy ,non-diabetic renal disease and for renal effects of various drugs, hormones, and nephrotoxins. Urinary tract infections and congestive heart disease are also possible causes of elevations in the albumin extraction rate. (6-10)

II.Principle of the test

The Microalbumin quantitative test kit is based on solid phase enzyme immunoassay (EIA). This assay system uses two antibodies (a rabbit polyclonal antibody and a mouse monoclonal antibody) directed against distinct antigenic determinants on the Albumin molecule. The polystyrene wells are coated with captured rabbit polyclonal antibodies against Albumin.Standards,controls and patient samples are added to the wells (solide phase) and incubate. The Albumin present in the samples is bound to the anti-Albumin antibodies. The unbound material is removed by aspiration and washing. After washing, the HRP labeled anti-Albumin (Mab) is added to the wells. After second incubation and washing, a solution of TMB (3,3',5,5' tetra-methylbenzidine) is added to each well, resulting in the development of a blue color. The intensity of the color is inversely proportional to the amount of albumin present in the sample. The color development is stopped by addition of Stop solution, causing the blue color to change to yellow. The color intensity is determined in a microtiter plate spectrophotometer at 450 nm. Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard. The Albumin concentrations of patient samples are then read from the standard curve.

III.Kit contents

The reagents provided with the Microalbumin Kits (Cat.No.P-MAI) are sufficient for 96 wells. The expiry date of each reagent is shown on the vial label.

Store the Kit at 2-8° C.

1.Coated microtiter wells: 96 wells,coated with rabbit polyclonal anti-Albumin antibodies.

2.Standards: 7 vials (1.0 ml) of human Albumin in matrix with thimerosal as a preservative .The exact Albumin concentration for each standard is specified on the label of each vial.

3.Microalbumin controls: 2 vials (1.0 ml) of human Albumin in processed human serum containing thimerosal. The nominal Albumin concentrations in the controls are specified on the label of each vial.

4.Enzyme tracer: 1 vial (12 ml) of monoclonal anti- Albumin antibody conjugated to horseradish peroxidase (HRP) in phosphate buffered saline (PBS) with proteins and thimerosal.

5.Wash solution (concentrate 20X): 1 vial (12 ml) of PBS-Tween 20 and thimerosal.

6.TMB HRP-Substrate: 1 vial (12 ml) of buffered H_20_2 and 3,3',5,5' tetra methylbenzidine.

7.Stop solution: 1 vial (12ml) of 2N H_2S0_4.

IV.Materials required but not supplied with the kit

1.Microtiter plate spectrophotometer reader with a wavelength of 450 nm(with reference wavelength at 630 nm) and an absorbance range of 0 to 3.0.

2.Precision micropipettes with disposable plastic tips to deliver 25- 100 µl.

3. Distilled or deionized water for preparation of diluted Wash Solution.

V. Specimen collection and preparation

The assay can be performed in urine collected without preservative .Urine samples must not be contaminated with protein from non-urinary source (blood or semen). Urine samples should not be obtained following an exercise period. Samples with a pH of less than 4 or greater than 8 may yield result which are too high or too low respectively .The test should not be performed if the sample exhibits significant bacterial growth or if the patient shows signs of a urinary tract infection. (11-12)

Keep samples at 2-8°C for 1 week; for longer periods it is recommended to store the sample in aliquot form at -20°C. Avoid repeated freezing and thawing of samples. Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand.
Do not vortex patient samples.
Urine sample with Microalbumin greater than the last standards should be diluted with dilution buffer and reassayed to give a quantitative result. The value obtained must be multiplied by the dilution factor to give the correct Microalbumin concentration.

VI. Assay procedures

All reagents should be brought to room temperature prior to use.

Dilute one part concentrated wash solution with 19 parts distilled or deionized water to achieve working strength. Diluted wash solution is stable for 7 days at 2 to 8°C. In presence of undissolved crystals, re-suspend the solution by placing the vial at 37°C for a few minutes.

1. Dispense 25 µl of Microalbumin standards, control and patient samples into appropriate wells. Pipette 100 µl of Enzyme Tracer to each well .Thoroughly mix the plate for 60 seconds.

2. Incubate the strips for 60 minutes at room temperature.

3.Aspirate and wash each well 4 times with 300 µl of diluted Wash solution.

4. Dispense 100 µl of TMB HRP-Substrate into each well.

5. Incubate at room temperature in the dark for 15 minutes.

6. Add 100 µl of Stop Solution and mix for 10 seconds. Read absorbance at 450 nm (with reference wavelength at 630 nm) in a microtiter plate reader within 15 minutes after addition of Stop solution.

SUMMARY OF ASSAY PROCEDURE

Pipette 25 µl standard, control or sample

Pipette 100 µl Enzyme Tracer

Incubate 60 min at RT

Wash 4 x (300 µl)

Pipette 100 µl TMB

Incubate 15 min at RT

Pipette 100 µl Stop Solution

Read at 450/630 nm

VII. Calculation of results

The results can be calculated by either microtiter plate, spectrophotometer reader or manual evaluation. If a microtiter plate spectrophotometer reader with data calculation program is used, refer to the plate reader and create a program using the concentration of each of the Microalbumin standards (in µg/ml).
For manual evaluation, a standard curve is constructed by plotting the absorbance (A) values obtained for each Microalbumin standard against the corresponding Microalbumin concentrations (in µg/mll).The unknown Microalbumin concentration, in µg/ml, can then be read from the standard curve using the absorbance value of each patient specimen.

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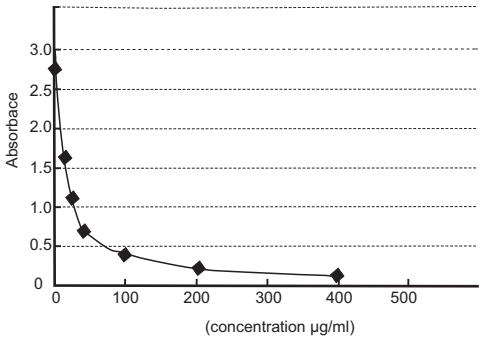
Example of calculation:

The values shown below are examples and must not be used in place of experimental data.

Specimen	Albumin concentration (µg/ml)	Absorbance
Standard A	2.72	0
Standard B	1.64	10
Standard C	1.06	20
Standard D	0.67	40
Standard E	0.39	100
Standard F	0.22	200
Standard G	0.13	400
Sample	0.60	45



Reporting results:

Results are usually reported as excretion rate, micrograms of albumin per minute (µg/min) . This is obtained by multiplying the concentration in µg/ml,as read from the curve, by the dilution factor and the by the total volume , in ml, and the dividing by the duration of the collection (in minutes)

$$\frac{\text{(urinary albumin concentration in µg/ml) x (sample volume in ml)}}{\text{(collection interval in minutes)}}$$

Urine albumin concentration , obtained in µg/ml , can be converted to µg/min for timed fractional , overnight or 24-hour collections.

VIII. Normal values

In general, the normal range of albumin concentration in urine is 1.0-20.0 µg/ml.To compare our own results with published normal values for urinary albumin excretion , urine samples from 117 apparently healthy individual were analyzed(<50 years old) .This random screening showed that more than 98% of the individuals had normal levels of albumin(<20 µg/ml)[12-14].
The above values however are only indicative . We suggest that each laboratory determine its own normal values for the interpretation of patient results.

IX. Specific assay characteristics

1. Sensitivity:The sensitivity of this kit was calculated based upon the standard curve and expressed as the minimal concentration of Albumin showing a significant difference from the dilution buffer. This minimal detectable concentration of Albumin is 0.5 µg/ml.

2. Hook effect: Samples with very high antigen concentration are tested undiluted. With this kit no hook effect has been noticed with samples containing Albumin concentration up to 150 mg/ml.

3.Comparison with Microalbumin Nephelometry method: Correlation studies on more than 500 random samples, were performed using the quantitative results from the Nephelometry. The correlation coefficient of test results was 0.97.

4.Precision: Precision was evaluated for intra- and inter-assay variability. Intra-assay reproducibility was determined by measurement of 10 replicates of 4 samples in a single run. Inter-assay reproducibility was determined by replicate measurement of 4 samples in 10 separate runs.

Reproducibility

	Intra-assay			Inter-assay		
	Mean(µg/ml)	SD	%CV	Mean(µg/ml)	SD	%CV
Sample 1	12.7	0.61	4.84	12.8	0.70	5.51
Sample 2	19	0.62	3.28	18.9	0.53	2.81
Sample 3	70	6.31	9.01	66.7	4.37	6.55
Sample 4	160.9	8.15	5.07	155.6	7.31	4.7

5.Recovery and dilution: Recovery is defined as the increase in concentration seen when a known concentration of an analyte is added to a sample. Spiked Samples were prepared by mixing the appropriate aliquot of a concentrated Albumin solution with a sample containing a low Albumin concentration. The percentage recovery is calculated as:

[(Measured conc. (µg/ml)/Expected conc. (µg/ml)] X100

The percentage recovery ranged from 96 to 110.

Recovery Test

Added Conc. (µg/ml)	Expected Conc. (µg/ml)	Measured Conc. (µg/ml)	% Recovery
-	-	22.8	-
8.8	92.3	98.7	107
17.6	142	155.8	110
35.2	208.2	200.6	96

For dilution test, several urine samples containing elevated Albumin concentrations were tested after serially diluting with the dilution buffer. The results of one of these samples are shown in the following table. The percentage recovery ranged from 103 to 107.5 .

Dilution test

Dilution	Expected Conc. (µg/ml)	Measured Conc. (µg/ml)	% Recovery
undiluted	-	362	-
1:2	181	187.9	103.8
1:4	90.5	93.2	103
1:8	45.2	48.6	107.5

6.Specificity : The addition of each of the following compounds to urine samples does not interfere with the measuring of albumin by using PADTAN ELM MicroAlbumin Kit :

Ovalbumin	1 mg/dl
Rat serum Albumin	1 mg/dl
Mouse serum Albumin	1 mg/dl
Bovine serum Albumin	1 mg/dl
CRP	5 mg/dl
Transferrin	2 mg/dl

X. References

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WARNING!

This kit contains toxic materials, animal and human sourced components. Since no method can completely rule out the presence of blood-borne disease(e.g. HIV,HCV and HBV) therefore,all human sourced material must be considered potentially infectious.
In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary. Never pipette by month. Do not eat, smoke or apply cosmetics in the laboratory.

Rev: Jul.2017

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