DRG

DRG® Microalbumin ELISA (EIA-3881)

CE

Revised 16 May 2008 (Vers. 1.1) IVD

NAME AND INTENDED USE

Micro-Albumin is a competitive solid phase enzyme immunoassay (ELISA) for the quantitative measurement of human albumin in urine. The assay is intended for in vitro use only as an aid in the determination of Microalbuminuria.

SUMMARY AND EXPLANATION OF THE TEST

Proteins passing the glomerular basal membrane of the kidney undergo differentiated filtering. The permeability is inversely proportional to the molecular weight (Albumin about 0.6 %, Myoglobulin about 75 %). Nevertheless, only minimal quantities of protein are detectable in urine, because big quantities of protein are reabsorbed by the tubuli. Elevated glomerular protein permeability and high tubular plasma protein elimination can be differentiated by measuring the molecular weight distribution of the eliminated proteins.

The pattern of eliminated proteins in urine give information about :

- elevated protein elimination
- prediagnosis of a kidney defect
- differentiation of proteinuria
- glomerular or tubular proteinuria

Diagnostically relevant proteins:

- IgG (150 kD)
- Alpha-1-Microglobulin (33 kD)
- Beta-2-Microglobulin (12 kD)
- Albumin (66 kD)
- Retinol binding protein (21 kD)
- Immunoglobulin light chains (Bence-Jones protein) (22 kD)

Albumin has a relative molecular mass of 66 kD. It is contained in urine at very low concentrations. In case of a very active glomerular filtering process the albumin secretion can arise without an underlying kidney disease. This situation is called "Microalbuminuria". The detection of these small secretion quantities (30 to 150 µg/min or ml) requires very sensitive test-systems1,6, i.e. immunological techniques. Physical stress can induce elevated albumin secretion too, without the occurence of a kidney disease.
In Diabetes, Albumin secretion is a very important parameter for the evaluation of the kidney function. Urine values higher than 25 µg/ml indicate a detrimental kidney function of insulin-dependent2,3 (type I) and non-insulin-dependent4,5,6 (type II) diabetic patients. The determination of Albumin is therefore an important diagnostic tool in diabetic nephropathies1,7,8.

Indication : Microalbuminuria

PRINCIPLE OF THE TEST

Highly purified human albumin is bound to microwells. Calibrators, controls and undiluted patient samples are pipetted together with anti-human-Albumin-peroxidase conjugate in the wells. Micro-albumin, if present in diluted urine, will compete with coated albumin for binding of the anti-albumin-conjugate. Washing of the microwells removes unreactive serum components. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is inversely proportional to the concentration of albumin present in the original sample.

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WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg and HIV by FDA approved methods. No test can guarantee the absence of HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which contains hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations, though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.)
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not Eat, Drink, Smoke or Apply Makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible **microplate** consisting of 12 modules of 8 wells each, coated with highly purified human albumin. Ready to use.
6 vials, 0.5 ml each	Micro-Albumin-**Standards** (A-F) in a PBS/BSA matrix (NaN_3 <0,1% (w/w)) containing micro-albumin: 0.15; 1.5; 6; 25; 100 and 400 µg/ml. Ready to use
2 vials, 0.5 ml each	Micro-Albumin **Controls** in a PBS/BSA matrix (NaN_3 <0,1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use
1 vial, 10 ml	**Sample Buffer** (Tris, NaN_3 <0,1% (w/w)), yellow. Ready to use
1 vial, 15 ml	**Enzyme conjugate** solution (PBS, PROCLIN 300 <0,5% (v/v)), (light red) containing polyclonal rabbit anti-human albumin; labelled with horseradish peroxidase. Ready to use
1 vial, 15 ml	**TMB Substrate Solution**. Ready to use
1 vial, 15 ml	**Stop Solution** (1 M hydrochloric acid). Ready to use
1 vial, 20 ml	**Wash Solution** (PBS, NaN_3 <0,1% (w/w)), concentrate (50x)

STORAGE AND STABILITY

1. Store the kit at 2-8°C
2. Keep microplate wells sealed in a dry bag with desiccants
3. The reagents are stable until expiration of the kit
4. Do not expose test reagents to heat, sun or strong light during storage and usage
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8°C

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- data reduction software

Preparation of reagents

- distilled or deionized water
- graduated cylinder for 100 and 1000 ml
- plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

For determination of Albumin urine is the preferred sample matrix.
Urine samples need not to be diluted. If higher concentrations are expected dilute samples with sample buffer.
The dilutions have to be considered during calculation.

The patients need not to be fasting, and no special preparations are necessary. Collect morning urine.
Samples may be stored refrigerated at 2 - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C.
To avoid repeated thawing and freezing the samples should be aliquoted.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates
2. Do not interchange kit components from different lots
3. All materials must be at room temperature (20-28°C)
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.

5. Perform the assay steps only in the order indicated
6. Always use fresh sample dilutions
7. Pipette all reagents and samples into the bottom of the wells
8. To avoid carryover contamination change the tip between samples and different kit controls
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and patient samples in duplicates.
2. Pipet 20 µl of standards, controls and undiluted patient samples into the wells.
3. Add 100 µl of enzyme conjugate solution into each well.
4. Incubate for 30 minutes at room temperature (20 - 28 °C).
5. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
6. Dispense 100 µl of TMB substrate solution into each well.
7. Incubate for 15 minutes at room temperature.
8. Add 100 µl of stop solution to each well of the modules at leave untouched for 5 minutes.
9. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600 - 690 nm is recommended.
   The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation

The Micro-Albumin ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Standard A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not met, the results are invalid and the test should be repeated.

Calculation of results

For the Micro-Albumin test a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended. Smoothed Spline approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation. The dilution of urine samples have to be considered.

Interpretation of results

In a normal range study with urine samples from healthy donors the following range has been established with the Micro-Albumin test:

urine samples: 0 - 25 µg/ml Albumin

It is recommended that each laboratory establishes its own normal and pathological ranges of urine levels.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments urine samples with high Albumin concentrations were diluted with sample buffer and assayed in the Micro-Albumin kit. The assay showed linearity over the full measuring range.

Precision (Reproducibility)

Statistics were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations each:

Intra-Assay		
Sample No	Mean [µg/ml]	CV [%]
1	25.2	5.3
2	50.9	3.3
3	80.2	3.6

Inter-Assay		
Sample No	Mean [µg/ml]	CV [%]
1	24.8	4.2
2	50.1	5.1
3	78.6	2.9

Sensitivity

The lower detection limit for Micro-Albumin has been determined at 0.5 µg/ml.

Specificity

The antisera (polyclonal, rabbit) labelled with horseradish peroxidase is highly specific for human Albumin.

LIMITATIONS OF PROCEDURE

The Micro-Albumin ELISA is a diagnostic aid and by itself is not diagnostic. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

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