DRG® Microalbumin ELISA (EIA-3881)

Revised 28 Oct. 2013 rm (Vers. 4.1) **IVD**

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

Micro-Albumin ELISA is a test system for the quantitative measurement human Albumin in urine.
This product is intended for professional in vitro diagnostic use only.

PRINCIPLE OF THE TEST

Highly purified human albumin is bound to microwells.
The reaction is based on a competitive ELISA method with these steps:
Calibrators, controls and urine samples are incubated together with anti-albumin-peroxidase conjugate in the wells. Albumin, if present, will compete with coated albumin for binding of the anti-albumin-conjugate. Washing of the microwells removes unspecific components. Bound enzyme conjugate will hydrolyze the enzyme substrate TMB. The addition of acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm.
The amount of colour is inversely proportional to the concentration of albumin present in the original sample.

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
- differentiation of proteinuria
- prediagnosis of a kidney defect
- glomerular or tubular proteinuria

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

Albumin has a relative molecular mass of 66 kDa. 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 systems [1,6], i.e. immunological techniques. Physical stress can also induce elevated albumin secretion, without the occurrence of a kidney disease.

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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 dependent [2, 3] (type I) and non-insulin-dependent [4,5,6] (type II) diabetic patients. The determination of albumin is therefore an important diagnostic tool in diabetic nephropathies [1, 7, 8].

Indication : Microalbuminuria

CONTENTS OF THE KIT

	Sufficient for 96 determinations
1	One divisible ***microplate*** consisting of 12 modules of 8 wells each. Ready to use.
1x 0.5 mL	***Calibrator A*** 0.15 µg/mL, containing serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
1x 0.5 mL	***Calibrator B*** 1.5 µg/mL, containing albumin in a serum/buffer matrix (PBS, detergent, NaN_3 0.09%), yellow. Ready to use.
1x 0.5 mL	***Calibrator C*** 6 µg/mL, containing albumin in a serum/buffer matrix (PBS, detergent, NaN_3 0.09%), yellow. Ready to use.
1x 0.5 mL	***Calibrator D*** 25 µg/mL, containing albumin in a serum/buffer matrix (PBS, detergent, NaN_3 0.09%), yellow. Ready to use.
1x 0.5 mL	***Calibrator E*** 100 µg/mL, containing albumin in a serum/buffer matrix (PBS, detergent, NaN_3 0.09%), yellow. Ready to use.
1x 0.5 mL	***Calibrator F*** 400 µg/mL, containing albumin in a serum/buffer matrix (PBS, detergent, NaN_3 0.09%), yellow. Ready to use.
1x 0.5 mL	***Control positive***, containing albumin in a serum/buffer matrix (PBS, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
1x 0.5 mL	***Control negative***, containing albumin in a serum/buffer matrix (PBS, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
20 mL	***Sample Buffer PA***, containing PBS, detergent, preservative sodium azide 0.09%, yellow. Ready to use.
15 mL	***Enzyme Conjugate***; containing anti-human albumin antibodies, HRP labelled; PBS, BSA, detergent, preservative Proclin 0.05%, light red. Ready to use.
15 mL	***TMB Substrate***; containing 3,3', 5,5'-Tetramethylbenzidin, colorless. Ready to use.
15 mL	***Stop Solution***; contains acid. Ready to use.
20 mL	***Wash Buffer***, containing Tris, detergent, preservative sodium azide 0.09%; 50X conc.
1	Instruction for Use
1	Certificate of Analysis

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MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µL
- Vortex mixer
- Pipettes for 10 µL, 100 µL and 1000 µL
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 mL and 100 mL
- Plastic container for storage of the wash solution

This ELISA is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect morning urine.
- Samples may be refrigerated at 2 °C - 8 °C for up to five days or stored at -20 °C up to six months.
- Avoid repetitive freezing and thawing of urine samples.

STORAGE AND STABILITY

- Store test kit at 2 °C - 8 °C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Shelf life of the unopened test kit is 18 months from day of production.
  Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2 °C - 8 °C.
  We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20 °C - 28 °C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.

- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:
  Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection:
  Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid:
  Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

Wash Buffer
Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 mL prior to use.

Sample Buffer PA
Sample buffer PA is ready to use.

Preparation of samples
Use undiluted urine sample.
If very high concentrations are expected, urine samples should be diluted with sample buffer and dilutions considered during calculation.

Note: Calibrators / Controls are ready to use and need not to be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **20 µL** of calibrators, controls and patient samples into the wells.
2. Dispense **100 µL** of enzyme conjugate into each well.
3. Incubate for **30 minutes** at room temperature (20 °C - 28 °C).
4. Discard the contents of the microwells and **wash 3 times with 300 µL** of wash solution.
5. Dispense **100 µL** of TMB substrate solution into each well.
6. Incubate for **15 minutes** at room temperature
7. Add **100 µL** of stop solution to each well of the modules
8. Incubate for **5 minutes** at room temperature.
9. **Read** the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D	P4										
E	E	P5										
F	F	P6										
G	C+	P7										
H	C-	P8										

P1, ... patient sample, A-F calibrators, C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Measuring range

The calculation range of this ELISA assay is 1.5 - 400 µg/mL

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay:
Cut-off 0 - 25 µg/mL

Interpretation of results

Negative: < 25 µg/mL
Positive: ≥ 25 µg/mL

Linearity

Patient samples containing high levels of albumin were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample .	Dilution .	Observed [µg/mL]	Expected [µg/mL]	O/E [%]
1	1:1	368.0	368.0	100
	1:2	186.0	184.0	101
	1:4	90.8	92.0	99
	1:8	45.2	46.0	98
	1:16	22.1	23.0	96
2	1:1	280.0	280.0	100
	1:2	143.4	140.0	102
	1:4	71.9	70.0	103
	1:8	33.8	35.0	97
	1:16	17.7	17.5	101

Limit of detection

Functional sensitivity was determined to be: 0.5 µg/mL

Reproducibility

Intra-assay precision:

Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision:

Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean [µg/mL]	CV [%]
1	25.2	5.3
2	50.9	3.3
3	80.2	3.6

Inter-Assay		
Sample	Mean [µg/mL]	CV [%]
1	24.8	7.2
2	50.1	5.1
3	78.6	2.9

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. 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 concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Bretzel, R.G. Hypertonie, Mikroalbuminurie und Insulinresistenz bei Diabetes mellitus. Wiener Klin. Wochenschr. 1994; 106: 774 - 792.
2. Diabetes and Endocrine Unit, Dudley Road Hospital, Birmingham, UK and Division of Community Health, Medical School, Guy`s Hospital, London, UK. Microalbuminuria in type I diabetic patients. Prevalence and clinical characteristics. Microalbuminuria Collaborative Study Group. Diabetes Care 1992; 15: 495 - 501.
3. Walker, J.D. et al. Glomerular structure in type-1 (insulin-dependent) diabetic patients with normo microalbuminuria. Kidn. Int. 1992; 41: 741 - 748.
4. Mogensen, C.E. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. New England J.Med. 1984; 310: 356 - 360.
5. Gall, M.A. et al. Prevalence of micro- and macroalbuminuria, arterial hypertension, retinopathy and large vessel disease in European type 2 (non-insulindependent) diabetic patients. Diabetologia 1991; 34: 655 - 661.
6. Bashyam, M.M. et al. Microalbuminuria in NIDDM. Diabetes Care 1993; 16: 634 - 635.
7. Caduff, F. et al. Erfassung der Mikroalbuminurie als Marker für die beginnende diabetische Nephropathie in der Praxis: welche Urinsammelmethode? Schweizerische Med. Wochenschr. 1991; 121: 324 - 331.
8. Ruilope, L.M. et al. Randomly allocated study of the effects of standard therapy versus ACE inhibition on micro-albuminuria in essential hypertension. J. Hypert. Suppl. 1994;12: S59 -S63.

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