DRG® Microglobulin alpha-1 (EIA-5100)

USA: RUO

Revised 2 Feb. 2011 rm (Vers. 2.1)

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

This kit is intended for Research Use Only.

This kit is not intended for diagnostic purposes.

1 INTENDED USE

The Assay is intended for determination of α1-Microglobulin in serum, plasma and urine.

2 TEST PRINCIPLE

This Enzyme-Linked Immunosorbent Assay (ELISA) allows the quantitative determination of human α_1-Microglobulin from plasma, serum and urine.

The α1-Microglobulin in the samples is bound to an excess of polyclonal rabbit anti-α1-Microglobulin antibodies immobilized to the surface of the microtitre plate.

After a washing step to remove all foreign substances, the quantification of the bound α1-Microglobulin is carried out by adding a peroxidase labeled antibody, which also binds to the α1-Microglobulin. The amount of converted peroxidase substrate is directly proportional to the amount of bound α1-Microglobulin and can be determined photometrically at 450 nm or at 405 nm if the extinction is out of range.

3 MATERIAL SUPPLIED

Content	Kit Components	Quantity
PLATE	one holder with strips, break apart	96
WASHBUF	ELISA wash buffer concentrate (10x)	1 x 100 ml
CONJ	Conjugate (rabbit-anti-α1 microglobulin)	1 x 400 µl
STD	Calibrators, (0, 0.019, 0.055, 0.166, 0.5, 1.5 ng/ml)	6 x 250 µl
CTRL 1	Control, lyophilized	1 x 250 µl
CTRL 2	Control, lyophilized	1 x 250 µl
NACL	0.9 % NaCl-solution, ready-to-use	25 ml
SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	2 x 15 ml
STOP	ELISA stop solution, ready-to-use	1 x 15 ml

4 MATERIAL REQUIRED BUT NOT SUPPLIED

Bidistilled (aqua bidest.) and sterile water
Laboratory balance

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Precision pipettors calibrated and tips to deliver 5-1000 µl
Foil to cover the microtiter plate
Horizontal microtiter plate shaker
A multi-channel dispenser or repeating dispenser
Centrifuge capable of 3000 x g
Vortex-Mixer
Standard laboratory glass or plastic vials, cups, etc.
Microtiter plate reader at 450 or 405 nm
(reference wave length 620 or 690 nm)

5 PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- The **WASHBUF** (wash buffer concentrate) should be diluted with aqua dest. **1:10** before use (100 ml WASHBUF + 900 ml aqua dest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature at 37°C using a water bath **before dilution of the buffer solutions**.
  The **WASHBUF** (wash buffer concentrate) is stable at **2-8°C** until the expiry date stated on the label.
  Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.
- The **STD** (Calibrators) and the **CTRL** (controls) must be reconstituted with **250 µl** aqua dest. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution.
  Reconstituted calibrators and controls can be stored at –20 °C until the expiry date given on the label. Repeated thawing and freezing should be avoided.
- The **CONJ** (conjugate, POD-Antibody) must be diluted **1:100** in ELISA wash buffer (e.g. 200 µl CONJ + 20 ml wash buffer).
  The antibody is stable at 2 -8 °C until expiry date given on the label. **Diluted antibody is not stable and could not be stored.**

6 PRECAUTIONS

- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on the kit label.

7 SPECIMEN COLLECTION AND PREPARATION

Plasma or serum

Samples can be stored for two weeks at 4°C. They should be frozen when stored longer.

Dilute all plasma and serum samples **1:500 with NACL (0.9% NaCl)**
(e.g. 10 µl sample + 990 µl 0.9% NaCl = 1:100 dilution;
and then 100 µl from the 1:100 dilution + 400 µl 0.9% NaCl).

Urine

Urine should be adjusted to a pH of 6 to 8 with 1 N NaOH. Adjusted samples can be stored at 2-8 °C for 14 days. For longer storage, non-treated samples should be frozen at -20 °C.
Dilute all urine samples **1:20 with 1% BSA in PBS** (e.g. 50 µl urine + 950 µl 1% BSA in PBS)**.**

8 ASSAY PROCEDURE

8.1 Procedural notes

- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. DRG can therefore not be held responsible for any damage resulting from wrong use.
- The assay should always be performed according the enclosed manual.

8.2 Test procedure

Carry out the tests in duplicate in the supplied microtitre plate.

1. Wash the cavities **5 x with 250 µl** of washing buffer.
2. Add **200 µl of NACL** (0.9% NaCl solution).
3. Add **10 µl** of **STD** (standard), **CTRL** (controls) and **sample** into each well in duplicate
4. Cover the plate tightly and incubate for **1 hour at room temperature (18 °C – 26 °C) shaking** on a horizontal mixer or for **2 hours at room temperature (18 °C – 26 °C) without any shaking**.
5. Decant the contents of the wells and wash the cavities **5x with 250 µl** of washing buffer.
6. Add **200 µl pre-diluted conjugate**.
7. Incubate for **1 hour** shaking on a horizontal mixer at room temperature.
8. Decant the contents of the wells and wash the cavities **5x with 250 µl** of washing buffer.
9. Add **200 µl SUB** (TMB-substrate solution).
10. Incubate for **10 - 20 minutes** at room temperature until colour differences are sufficient.
11. Add **50 µl of STOP** (stop solution) and mix shortly.

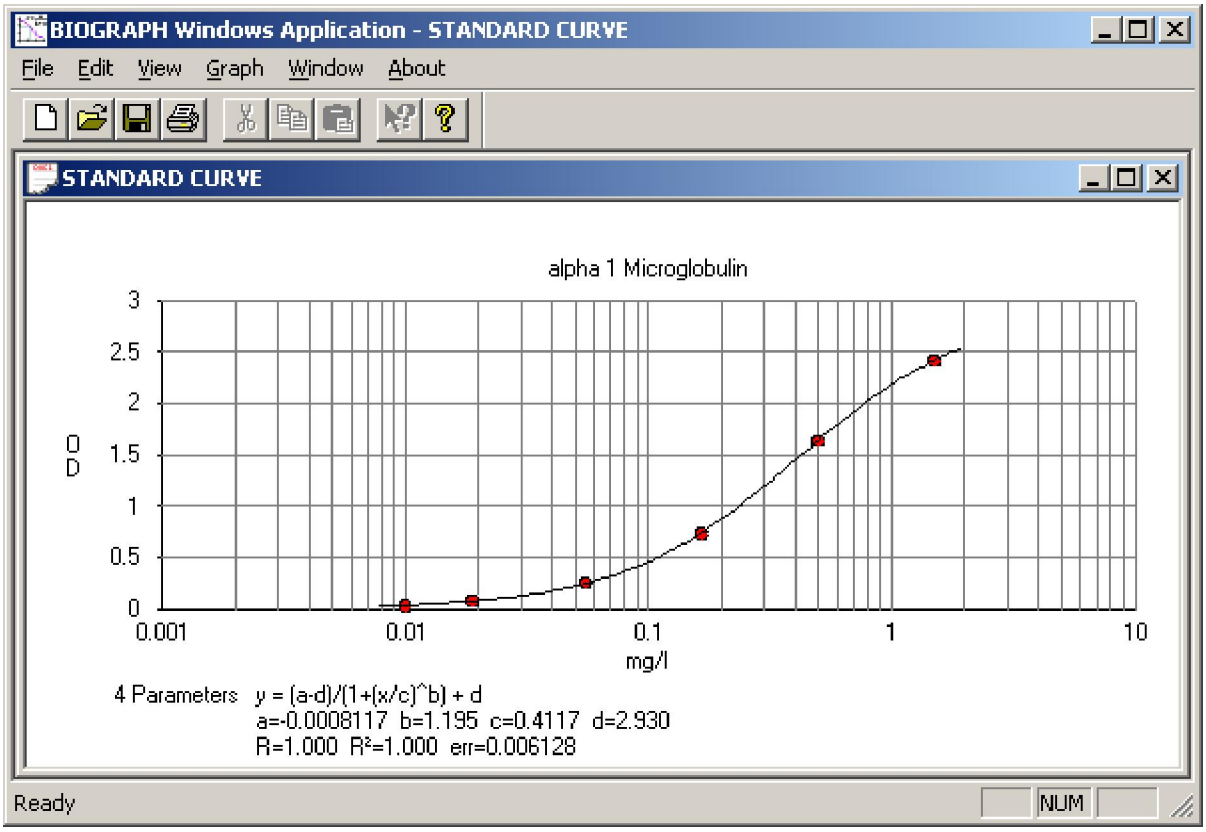
12. Determine **immediately** absorption with an ELISA reader at **450 nm** against 620 nm as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.

9 RESULTS

A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve.

THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline- or 4PL algorithm is recommended.

9.1 Typical calibration curve



Concentration [mg/l]	0	0.019	0.055	0.166	0.5	1.5
OD mean value	0.025	0.076	0.250	0.732	1.638	2.414

The data is for demonstration only and cannot be used for the evaluation of test results.

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Serum, Plasma

The result must be multiplied by **500** to calculate the concentration of the sample.

Urine

The result must be multiplied by **20** to calculate the concentration of the sample.

10 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.

Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

All reagents in the kit package are for research only.

Reagents should not be used beyond the expiration date shown on the kit label.

Do not interchange different lot numbers of any kit component within the same assay.

Guidelines for medical laboratories should be observed.

Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. DRG can therefore not be held responsible for any damage resulting from wrong use.

Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to DRG together with a written complaint.