DRG® Calprotectin (Serum) ELISA (EIA-5111)

As of 29 Sept. 2010 rm (Vers. 2.1) **USA: RUO**

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

1 INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for determination of ***Calprotectin*** (MRP8/14) in serum, plasma and urine. It is for Research use only.

2 INTRODUCTION

Alternative names of calprotectin:
MRP8/14, L1, (p8,14), p34
Alternative names of the two proteins forming the heterocomplex calprotectin:
S100A8, Calgranulin A, MRP8 (Migration inhibition factor-related protein-8), CP-10 (in mouse)
S100A9, Calgranulin B, MRP14 (Migration inhibition factor-related protein-14)

3 MATERIAL SUPPLIED

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
AB	Detection antibody, (monoclonal anti-Calprotectin (MRP 8/14) antibody, biotinylated), concentrate	50 µl
STD	Calprotectin standards, lyophilized (0; 3.9; 15.6; 62.5; 250 ng/ml)	2 x 5 vials
CTRL 1	Control, lyophilized (see specification for range)	2 x 1 vial
CTRL 2	Control, lyophilized (see specification for range)	2 x 1 vial
CONJ	Conjugate, (extravidin peroxidase labeled), concentrate	50 µl
SUB	TMB substrate (Tetramethylbenzidine), ready to use	15 ml
STOP	ELISA stop solution, ready to use	15 ml

4 MATERIAL REQUIRED BUT NOT SUPPLIED

Bidistilled water (aqua bidest.)
Laboratory balance
Precision pipettors calibrated and tips to deliver 10-1000 µl
Covering foil for the microtiter plate
Horizontal microtiter plate shaker with 37 °C incubator
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 the 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 **ELISA wash buffer concentrate** (WASHBUF) should be diluted with aqua bidest. **1:10** before use (100 ml concentrate + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution of the buffer solutions. The **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 lyophilized **STD** (standards) and **CTRL** (controls) are stable at **2-8°C** until the expiry date stated on the label. The **STD** (standards) and **CTRL** (controls) must be reconstituted with **500 µl** aqua bidest. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted standards and control can be stored at 2-8°C for four weeks.**

The **detection antibody** (AB) must be diluted **1:1000** in wash buffer (WASHBUF)
(10 µl AB + 10 ml WASHBUF).
The antibody is stable at **2-8 °C** until expiry date given on the label.
Diluted antibody solution is not stable and could not be stored.

The **conjugate** (CONJ) must be diluted **1:1000** in wash buffer (WASHBUF)
(10 µl CONJ + 10 ml WASHBUF).
The antibody is stable at **2-8°C** until expiry date given on the label.
Diluted conjugate is not stable and can not be stored.

All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

6 SAMPLE PREPARATION

Serum and Plasma samples

Preanalytic handling

Significant differences in the calprotectin levels can be observed due to different sample preparation procedures, e. g. up to 10-fold higher serum levels compared to the plasma calprotectin concentrations. The reasons are as follows:

Granulocytes are activated during serum clotting and release granulocyte-activating markers. The time between serum collecting and analysis as well as repeated freeze-thaw cycles don't cause a calprotectin concentration shift.

On the contrary, in the case of plasma samples, varying the time between sampling and analysis or the number of freeze-thaw cycles will cause variation in the observed calprotectin levels. Therefore, the preanalytical conditions of plasma samples should be held constant. This is a general requirement independent of the used test-system.

The use of serum samples for calprotectin determinations is recommended.

Serum samples should be diluted **1:50** with ELISA-wash buffer before assaying.

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EDTA Plasma samples should be diluted **1:10** with ELISA-wash buffer before assaying.
Urine samples should be diluted **1:10** with ELISA-wash buffer before assaying.

7 ASSAY PROCEDURE

7.1 Principle of the test

The assay utilizes the two-site "sandwich" technique with two selected monoclonal antibodies that bind to human Calprotectin.

Standards, controls and diluted donor samples which are assayed for human Calprotectin are added to wells of microplate coated with a high affine monoclonal anti-human Calprotectin antibody. During the first incubation step, Calprotectin in the samples is bound by the immobilized antibody. In a next incubation step, a biotinylated monoclonal anti-human Calprotectin antibody is added to each microtiter well. Then a peroxidase labeled exravidin conjugate is added to each well and the following complex is formed: capture antibody - human Calprotectin – biotinylated detection antibody - Peroxidase conjugate. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the Calprotectin concentration of sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from standard. Calprotectin present in the donor samples, is determined directly from this curve.

7.2 Test procedure

1. Bring all reagents and samples to room temperature (18-26 °C) and mix well
2. Mark the positions of STD /SAMPLE/CTRL (Standards/Sample/Control) in duplicate on a protocol sheet
3. Take as many microtiter strips as needed from kit. Store unused strips covered at 2-8° C. Strips are stable until expiry date stated on the label
4. Wash each well 5 times by dispending 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper
5. Add 100 µl of STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well
6. Cover plate tightly and incubate for 1 hour at 37 °C on a horizontal mixer**
7. Aspirate the contents of each well. Wash 5 times by dispending 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper
8. Add 100 µl AB (detection antibody) into each well
9. Cover plate tightly and incubate for 1 hour at 37 °C on a horizontal mixer**
10. Aspirate the contents of each well. Wash 5 times by dispending 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper
11. Add 100 µl CONJ (conjugate) into each well
12. Cover plate tightly and incubate for 1 hour at 37 °C on a horizontal mixer**

13. Aspirate the contents of each well. Wash 5 times by dispending 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper
14. Add 100 µl of SUB (substrate) into each well
15. Incubate for 10 - 20 minutes at room temperature (18-26°C) in the dark*
16. Add 50 µl of STOP (stop solution) into each well, mix thoroughly
17. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference

*The intensity of the color change is temperature sensitive. We recommend to observe the procedure of the color change and to stop the reaction upon good differentiation.

**The above incubation steps at 37 °C on a horizontal mixer are recommended by the producer. If there is no possibility to incubate at 37 °C, while shaking, we recommend to incubate at 37 °C without any shaking.

8 RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm
   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).
2. Point-to-point-calculation
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.
3. Spline-algorithm
   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).
   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Serum

For calculation of calprotectin concentration in serum, the result must be multiplied by **50**.

EDTA Plasma

For calculation of calprotectin concentration in plasma, the result must be multiplied by **10**.

Urine

For calculation of calprotectin concentration in urine, the result must be multiplied by **10**.

9 PRECAUTIONS

For Resaerch use only.

Quality control guidelines should be observed.

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.

Stop solution contains 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.

10 REFERENCES

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4. Wilkinson MM, Busuttil A, Hayward C, Brock DJ, Dorin JR, Van H, V: **Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues.** *J Cell Sci* 1988, **91 (Pt 2):** 221-230.
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