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

Revised 7 April 2014 rm (Ver. 6.1) **USA: RUO**

This kit is intended for Research Use Only.

Not intended for diagnostic purposes.

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

1 INTENDED USE

The described Calprotectin ELISA kit is intended for measurement of (MRP8/14, S100A8/A9) in serum, EDTA-plasma and urine.

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)

Calprotectin is a calcium-binding protein secreted predominantly by neutrophils and monocytes. The heterocomplex consists of the two proteins, S100A8 (calgranulin A) and S100A9 (calgranulin B), also designated as MRP8 and MRP14, respectively.

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	Control, lyophilized (see specification for range)	2 x 1 vial
CTRL	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

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4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- 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 3000g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader

* DRG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity < 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

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 **1:10 in ultra pure water** before use (100 mL WASHBUF + 900 mL ultra pure water), 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 ultra pure water.** Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution.
Reconstituted standards and controls can be stored at 2 - 8 °C for four weeks.

The **detection antibody** (AB) must be diluted **1:1000** in wash buffer
(10 µL AB + 10 mL wash buffer).
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
(10 µL CONJ + 10 mL wash buffer).
The antibody is stable at **2 - 8 °C** until expiry date given on the label.
Diluted conjugate is not stable and can't 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 SPECIMEN COLLECTION AND PREPARATION

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.

Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.

Serum samples

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

Plasma samples

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

Urine samples

Urine samples should be diluted **1:10** with 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.

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Standards, controls and diluted 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 extravidin 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 samples, is determined directly from this curve.

7.2 Test procedure

1. Bring all **reagents and samples** to **room temperature** (15 - 30 °C) and mix well
2. Mark the positions of **STD /SAMPLE/CTRL** (Standards/Sample/Controls) 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. Add **100 µL of STD/SAMPLE/CTRL** (Standard/Sample/Controls) in duplicate into respective well
5. Cover plate tightly and incubate for **1 hour at 37 °C on a horizontal mixer****
6. Aspirate the contents of each well. Wash **5 times** by dispending **250 µL of wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper
7. Add **100 µL AB** (detection antibody) into each well
8. Cover plate tightly and incubate for **1 hour at 37 °C on a horizontal mixer****
9. Aspirate the contents of each well. Wash 5 times by dispending **250 µL of wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper
10. Add **100 µL CONJ** (conjugate) into each well
11. Cover plate tightly and incubate for **1 hour at 37 °C on a horizontal mixer****
12. Aspirate the contents of each well. Wash **5 times** by dispending **250 µL of wash buffer** into each well. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper
13. Add **100 µL of SUB** (substrate) into each well
14. Incubate for **10 - 20 minutes at room temperature (18 °C - 26 °C) in the dark***
15. Add **100 µL of STOP** (stop solution) into each well, mix thoroughly
16. Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If the extinction of the highest standards 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 linear abscissa for concentration.

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 the dilution factor of **50**.

EDTA-Plasma

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

Urine

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

9 LIMITATIONS

Samples with an OD greater than the OD of the highest calibrator should be further diluted and re-assayed.

10 QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

10.1 Expected values: Each Laboratory should develop its own expected range.

11 PRECAUTIONS

- Control samples should be analyzed with each run.

- 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 Proclin as bactericides. Sodium azide and Proclin are toxic.
- Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

12 TECHNICAL HINTS

Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not to assemble wells of different microtiter plates for analysis, even if they are of the same batch as wells from already opened microtiter plates are exposed to different conditions as sealed ones.

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

Substrate solution should remain colourless until use.

To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Avoid foaming when mixing reagents.

The assay should always be performed according the enclosed manual.

13 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Quality control guidelines should be followed.
- 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 should be send to DRG along with a written complaint.

14 REFERENCES

1. Striz I, Trebichavsky I: **Calprotectin - a pleiotropic molecule in acute and chronic inflammation.** *Physiol Res* 2004, **53:** 245-253.
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3. Odink K, Cerletti N, Bruggen J, Clerc RG, Tarcsay L, Zwadlo G *et al*.: **Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis.** *Nature* 1987, **330:** 80-82.
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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6. Hermani A et al. **Calcium-Binding Proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer.** *Clin Cancer Res* 2005, 11: 5146-5152.

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