DRG® S100A8 / S100A9 (Mouse/Rat) (EIA-5142)

As of 1 Mar. 2011 rm (Vers. 1.1) **USA: RUO**

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

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for determination of S100A8/S100A9 (Calprotectin, MRP (8/14) in stool, serum, plasma, urine, tissue extract and cell culture supernatant.

2 INTRODUCTION

Alternative names: Calgranulin A: MRP8, S100A8, CP-10
Calgranulin B: MRP14, S100A9,
Calprotectin, MRP8/14: L1, (p8,14), p34

3 MATERIAL SUPPLIED

Content	Kit Components	Quantity
PLATE	One holder with strips	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
EXBUF	Extraction buffer concentrate 2,5x	90 ml
AB	Detection antibody, (monoclonal anti-S100A8/S100A9 (MRP 8/14) antibody), lyophilized	450 µl
STD	S100A8/S100A9 standards, lyophilized (0; 0.25; 0.98; 3.9; 15.6 ng/ml)	2 x 5 vials
CTRL	Control, lyophilized (see specification for range)	2 x 1 vial
CONJ	Conjugate (anti-mouse, peroxidase labeled), concentrate	200 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 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 WASHBUF** (wash buffer concentrate) must be diluted with aqua bidist. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidist.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be at 37°C in a water bath before dilution.
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 **EXBUF** (extraction buffer concentrate) must be diluted with aqua bidist. **1:2.5** before use (90 ml EXBUF + 135 ml aqua bidist.), mix well.
Crystals could occur due to high salt concentration in the stock solutions.
Before dilution, the crystals must be redissolved at 37°C in a water bath.
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 three months**.

The lyophilized **STD** (standards) and **CTRL** (control) are stable at **2-8°C** until the expiry date stated on the label. The **STD** (standards) and **CTRL** (control) 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 lyophilized **AB** (detection antibody) is stable at **2-8°C** until the expiry date stated on the label.
The lyophilized **AB** (detection antibody) must be reconstituted with **450 µl diluted wash buffer**. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution.
The reconstituted detection antibody must be further diluted **1:400 in wash buffer** (25 µl reconstituted detection antibody + 10 ml wash buffer).
The reconstituted detection antibody is stable at -20°C up to 4 weeks.
Diluted detection antibody solution is not stable and can not be stored.

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The **CONJ** (conjugate) must be diluted **1:100** in **wash buffer**, e. g.:10 µl CONJ + 990 µl wash buffer, mix.
100 µl of diluted conjugate per well are used in the assay.
The undiluted conjugate is stable at **2-8 °C** until the expiry date stated 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 given on the label when stored at **2-8°C**.

6 SAMPLE PREPARATION

Stool samples

Each sample must be suspended **1:50** in extraction buffer and centrifuged for 5 minutes at 13000 g.

For analysis, pipette **100 µl** of the supernatant per well.

Serum samples

Samples should be diluted **1:100** with wash buffer before assaying.

For analysis, pipette **100 µl** of the dilution per well.

Urine samples

Samples should be diluted at least **1:3** with wash buffer before assaying.

For analysis, pipette **100 µl** of the dilution per well.

Cell culture supernatants

Samples should be diluted at least **1:2** with wash buffer before assaying.

For analysis, pipette **100 µl** of the dilution per well.

7 ASSAY PROCEDURE

7.1 Principle of the test

The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to S100A8/S100A9.

Standards, controls and diluted samples which are assayed for S100A8/S100A9 are added to wells of microplate coated with high affine anti-S100A8/S100A9 antibodies. During the first incubation step, S100A8/S100A9 in the samples is bound by the immobilized antibodies. In a next incubation step, a monoclonal anti-S100A8/S100A9 antibody is added to each microtiter well. Then a peroxidase labeled anti-mouse conjugate is pipetted into each well and the following complex is formed: capture antibodies - S100A8/S100A9 – 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 S100A8/S100A9 concentration of the sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. S100A8/S100A9 present in the 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** with **250 µl of diluted wash buffer**. 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 each well **5 times** with **250 µl of diluted wash buffer**. 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 each well **5 times** with **250 µl of diluted wash buffer**. 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 each well **5 times** with **250 µl of diluted wash buffer**. 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 **5 - 15 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.

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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. 0.01).
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. 0.01).

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.

Stool samples

To obtain the concentration, the result must be multiplied by **50**.

Serum samples

To obtain the concentration, the result must be multiplied by **100**.

Urine and Cell culture supernatants

To obtain the concentration, the result must be multiplied by the corresponding dilution factor.

The test results represent only relative values, as there are no data on the cross reactivity.

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9 PRECAUTIONS

For research 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 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.

10 TECHNICAL HINTS

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

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

Substrate solution should remain colorless 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.

11 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

All reagents in the kit package are for research use only.

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.

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