DRG® Calprotectin (Stool) (2h) (MRP 8/14) (EIA-5190)

CE

Revised 24 Mar.2011 rm (Vers. 2.1) **RUO in the USA**

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.

INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for determination of Calprotectin (MRP (8/14) in stool.

Alternative names:
Calgranulin A: MRP8, S100A8, CP-10 (in mouse)
Calgranulin B: MRP14, S100A9,

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
EXBUF	Extraction buffer concentrate 2,5x	2 x 90 ml
STD	Calprotectin standards, lyophilized (0; 13; 52; 210; 840 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	200 µl
SUB	TMB substrate (Tetramethylbenzidine), ready to use	15 ml
STOP	ELISA stop solution, ready to use	15 ml

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 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)

* 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 of 0.055 µS/cm at 25°C (≤18.2 MΩ cm).

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 WASHBU***F* (wash buffer concentrate) must be diluted with ultra pure water. **1:10** 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 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 ultra pure water. **1:2.5** before use (90 ml EXBUF + 135 ml ultra pure water.), 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** (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 **CONJ** (conjugate) must be diluted **1:100** in wash buffer (100 µl CONJ + 9.9 ml wash buffer).
  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**.

CE

Revised 24 Mar.2011 rm (Vers. 2.1) **RUO in the USA**

SAMPLE PREPARATION

Extraction of the stool sample

Diluted extraction buffer is used as a sample extraction buffer. We recommend the following sample preparation:

1a. Stool Sample Application System (SAS) (Cat. No.: 6998SAS)

Stool sample tube – Instruction for use

Please note that the dilution factor of the final stool suspension depends on the used amount of stool sample and the volume of the buffer.

SAS with 0.75 ml Buffer:

Applied amount of stool: 15 mg
Buffer Volume: 0.75 ml
Dilution Factor: 1:50

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a. The raw Stool Sample has to be thawed. For remarkably inhomogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.
b. **Fill the empty sample tube with 0.75 ml** of ready-to-use extraction buffer before using it with the sample. Important: Allow the extraction buffer to reach room temperature.
c. Unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into sample. The lower part of the dipstick exhibits notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off and leave 15 mg of sample to be diluted. Screw tightly to close the tube.
d. Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for app. 10 minutes improves the result.
e. Allow sample to stand for app. 10 minutes until sediment has settled down. Floating material like shells of grains can be neglected.
f. Carefully unscrew the complete cap of the tube including the turquoise ring plus the dipstick. Discard cap and dipstick. Make sure, the sediment will not be dispersed again.

1b. Sample preparation kit from Roche Diagnostics, Mannheim, Germany (Cat. No. 10 745 804 322)

Alternatively, other stool sample preparation kits (e.g. Sample preparation kit from Roche Diagnostics, Mannheim, Germany) can be used. In the Roche sample preparation kit, 100 mg of stool sample are suspended in 5 ml of extraction buffer using a vibrator mixer (e.g. Vortex mixer). Centrifugation of the suspension is recommended.

Dilution I (1a. or 1b.) 1:50

CE

Revised 24 Mar.2011 rm (Vers. 2.1) **RUO in the USA**

Dilution of samples

Stool samples

The suspension of sample preparation procedure 1a. or 1b. (dilution I)is diluted **1:50 with wash buffer.**
For example:
20 µl supernatant (dilution I) + 980 µl wash buffer = 1:50 **(dilution step II)**
For analysis, pipette **100 µl** of the supernatant of **dilution step II** per well.

Calprotectin in stool is described to be stable for approximately 6 days. Nevertheless, we recommend to store the samples for not more than 48 h at 2-8 °C. Long term storage is recommended at -20 °C. Allow frozen samples to thaw slowly, preferably at 2–8° C over night and warm the samples to room temperature before analysis.
(Poullis A et al. (2002) Aliment Pharmacol Thr 16:675-681)

ASSAY PROCEDURE

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 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. Then a peroxidase labeled conjugate is added to each well and the following complex is formed: capture antibody - human Calprotectin – 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) vs. concentration is generated, using the values obtained from standard. Calprotectin present in the samples, is determined directly from this curve.

DRG® Calprotectin (Stool) (2h) (MRP 8/14) (EIA-5190)

CE

Revised 24 Mar.2011 rm (Vers. 2.1) **RUO in the USA**

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/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. Wash each well **5 times** with **250 µl of 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/Controls) in duplicate into respective well
6. Cover plate tightly and **incubate for 1 hour at 37 °C** on a horizontal mixer**. Alternatively, incubate **overnight at room temperature** on a horizontal mixer
7. Aspirate the contents of each well. Wash each well **5 times** with **250 µl of wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper
8. Add **100 µl CONJ** (conjugate) 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 of SUB** (substrate) into each well
12. Incubate for **10–20 minutes at room temperature** (18-26°C) in the dark*
13. Add **50 µl of STOP** (stop solution) into each well, mix thoroughly
14. Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used

* 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.

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.

Stool samples
To obtain the calprotectin concentration in the stool samples,
multiply the obtained result by **2500** (dilution step I x dilution step II).

LIMITATIONS

Stool samples with Calprotectin levels greater than the highest standard value, should be diluted with wash buffer, and be re-assayed.

PRECAUTIONS

- 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.

CE

Revised 24 Mar.2011 rm (Vers. 2.1) **RUO in the USA**

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 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.

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- 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.

REFERENCES

1. Poullis A et al. (2002) Aliment Pharmacol Thr 16:675-681
2. Fagerhol et al. (2000) The Lancet 356:1783-1784
3. Tibble et al. (2000) Gut 47:506-513
4. Tibble et al. (2000) Gastro 119:15-22
5. Langhorst J, Koelzer J, Elsenbruch S, Rueffer A, Michalsen A, Dobos GJ (2007)
   Non-invasive Marker der Entzündungsaktivität bei Patienten mit chronisch entzündlichen Darmerkrankungen (CED): Vergleich von Lactoferrin, Calprotectin, PMN-Elastase im Stuhl, Serum-CRP und klinischen Aktivitätsindizes.
   Z Gastroenterol 45: P261
6. Schröder O, Naumann M, Shastri Y, Povse N, Stein J (2007)
   Prospective evaluation of faecal neutrophil-derived proteins in identifying intestinal inflammation: combination of parameters does not improve diagnostic accuracy of calprotectin. Aliment Pharmacol Ther Oct 1;26(7):1035-42
7. Shastri YM , Bergis D, Schäfer V, Povse N, Stein J (2006)
   Prospective Muliticentre double blind randomized controlled trial for predicting microbiological stool culture positivity for acute diarrhea. Poster presented at Conference of Indian Society of Gastroenterology, November 7-12, 2006, Mumbai