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immun diagnostik

Arbeitsanleitung / Manual

S100A8/S100A9 ELISA Kit

Zur Bestimmung von S100A8/S100A9 (Calprotectin, MRP 8/14) in Stuhl, Serum, Plasma, Urin, Gewebeextrakt, Zellkulturüberstand

Für Tier-experimentelle Studien (Maus, Ratte; nicht für humanes Probenmaterial geeignet)

S100A8/S100A9 ELISA Kit

For the determination of S100A8/S100A9 (Calprotectin, MRP 8/14) in stool, serum, plasma, urine, tissue extract, cell culture supernatant

For animal experimental studies (mouse, rat; not suitable for human samples)

Gültig ab / Valid from 02.09.2014

REF K 6936

Σ 96

+8°C +2°C

RUO

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Manual

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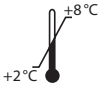

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Table of Contents

1. INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative determination of S100A8/S100A9 (Calprotectin, MRP (8/14) in stool, serum, plasma, urine, tissue extract and cell culture supernatant. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Alternative names:

Calgranulin A: MRP8, S100A8, CP-10

Calgranulin B: MRP14, S100A9,

Calprotectin, MRP8/14: L1, (p8,14), p34

S100A8/S100A9 (MRP (8/14) is a calcium-binding protein secreted predominantly by neutrophils and monocytes. Fecal S100A8/S100A9 is a marker for neoplasic and inflammatory gastrointestinal diseases.

It is often difficult to distinguish between irritable bowel syndrome and chronic inflammatory bowel disease. This leads in many cases to extensive and unnecessary colonoscopic examinations. The S100A8/S100A9 test allows clear differentiation between the two patient groups. Fecal S100A8/S100A9 levels correlate significantly with histological and endoscopic assessment of disease activity in Morbus Crohn's disease and ulcerative colitis as well as with the fecal excretion of indium-111-labelled neutrophilic granulocytes that has been suggested as the "gold standard" of disease activity in inflammatory bowel disease. However, measuring 111-indium-labeled granulocytes is very costly (patient's hospitalization, analysis and disposal of isotopic material) and is connected with radioactive exposition of the patients. For this reason, a repeated application to children and pregnant women is not recommended.

Elevated levels of S100A8/S100A9 are a much better predictor of relapse than standard inflammatory markers (CRP, ESR HB). Comparing this marker with standard fecal occult blood screening in colourectal cancer demonstrates clearly the diagnostic advantages of the fecal S100A8/S100A9 test. The parameter is of a high diagnostic value: If the S100A8/S100A9 level in stool is low, there is a high probability that an organic disease does not exist.

Indications

- Marker for acute inflammation
- Estimation of gastrointestinal inflammation degree

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6936MTP	PLATE	One holder with strips	12 x 8 wells
K 6936WP	WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
K 6936EP	EXBUF	Extraction buffer concentrate 2.5x	90 ml
K 6936A2	AB	Detection antibody, (monoclonal anti-S100A8/S100A9 (MRP 8/14) antibody), lyophilized	450 µl
K 6936ST	STD	S100A8/S100A9 standards, lyophilized (0; 0.25; 0.98; 3.9; 15.6 ng/ml)	2 x 5 vials
K 6936KO	CTRL	Controls, lyophilized (see specification for range)	2 x 1 vial
K 6936K	CONJ	Conjugate (anti-mouse, peroxidase labeled), concentrate	200 µl
K 6936TMB	SUB	TMB-Substrat (Tetramethylbenzidin), gebrauchsfertig	1 x 15 ml
K 6936AC	STOP	ELISA-Stopplösung, gebrauchsfertig	1 x 15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker with 37 °C incubator
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG 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).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** 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 concentrate + 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 37 °C 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** (wash buffer) can be stored in a closed flask at **2–8 °C for one month**.
- The **extraction buffer concentrate** (EXBUF) 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 standards** (STD) and **controls** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. Before use, the standards and controls must be reconstituted with **500 µl of ultra pure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to ensure complete reconstitution. **Reconstituted standards and controls can be stored at 2–8 °C for four weeks.**
- The **lyophilized detection antibody** (AB) is stable at **2–8°C** until the expiry date stated on the label. The **lyophilized detection antibody** (AB) 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 **1:401** 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.**
- The **conjugate concentrate** (CONJ) must be diluted **1:101 in wash buffer** (100 µl CONJ + 10 ml wash buffer). The concentrate is stable at **2–8 °C** until the

expiry date stated on the label. **Diluted conjugate is not stable and cannot be stored.**

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

6. PREPARATION OF SAMPLES

Feaces

Each sample must be extracted **1:50** in extraction buffer (e.g. 100 mg feaces + 5 ml extraction buffer), and then centrifuged for 10 minutes at 3000 *g*.

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

EDTA-Plasma/Serum

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

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

Urine

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

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 is used as a substrate for peroxidase. Finally, an acidic stop

solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour 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.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

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.

We recommend to carry out the tests in duplicate.

1.	Wash each well **5 x** by dispensing **250 µl of diluted WASHBUF** (wash buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
2.	Add **100 µl of STD** (Standard) **SAMPLE** (Sample) **CTRL** (Controls) into respective well.
3.	Cover the plate tightly and incubate for **1 hour** at **37°C** on a horizontal shaker.*
4.	Discard the contents of each well. Wash each well **5 x** by dispensing **250 µl of diluted WASHBUF** (wash buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
5.	Add **100 µl diluted AB** (detection antibody) into each well.
6.	Cover the plate tightly and incubate for **1 hour** at **37°C** on a horizontal shaker.*
7.	Discard the contents of each well. Wash each well **5 x** by dispensing **250 µl of diluted WASHBUF** (wash buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
8.	Add **100 µl diluted CONJ** (conjugate) into each well.
9.	Cover the plate tightly and incubate for **1 hour** at **37°C** on a horizontal shaker.*

10.	Discard the contents of each well. Wash each well **5 x** by dispensing **250 µl of diluted WASHBUF** (washbuffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
11.	Add **100 µl** of **SUB** (substrate) into each well.
12.	Incubate for **5–15 min**.** at room temperature (15-30°C) in the dark.
13.	Add **100 µl of STOP** (stop solution) into each well, mix.
14.	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 colour change is temperature sensitive. We recommend to observe the procedure of the colour change and to stop the reaction upon good differentiation.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Feaces

To obtain the concentration, the result must be multiplied with the dilution factor **50**.

EDTA-Plasma/Serum

To obtain the concentration, the result must be multiplied with the dilution factor **100**.

Urine

To obtain the concentration, the result must be multiplied with the dilution factor **3.**

Cell culture supernatants

To obtain the concentration, the result must be multiplied with the dilution factor **3.**

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

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

9. LIMITATIONS

Samples with concentrations above the measurement range must be further diluted and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × sample dilution factor to be used

10. QUALITY CONTROL

Immundiagnostik recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the

same assay one or more values of the quality control sample are outside the acceptable limits.

11. PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The Zero-standard was measured 20 times. The detection limit was set as B_0 + 3 SD and estimated to be 0.076 ng/ml.

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- 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 colour 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 up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- 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.

- Do not mix plugs and caps from different reagents.
- The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for medical laboratories 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. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

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Used symbols:

 Temperature limitation

REF Catalogue Number

RUO For research use only

 Contains sufficient for <n> tests

 Manufacturer

 Use by

LOT Lot number