Manual

S100A8/S100A9 ELISA

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)

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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
KR6936	PLATE	Holder with strips	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate 10x	2 x 100 ml
KR6936	EXBUF	Extraction buffer concentrate 2.5x	90 ml
KR6936	AB	Detection antibody concentrate, (monoclonal anti-S100A8/S100A9 antibody), lyophilised	250 µl
KR6936	STD	S100A8/S100A9 standards, lyophilised (0; 0.25; 0.98; 3.9; 15.6 ng/ml)	2 x 5 vials
KR6936	CTRL	Controls, lyophilised (see specification for range)	2 x 1 vial
KR6936	CONJ	Conjugate (anti-mouse, peroxidase labeled), concentrate	200 µl
KR0002.15	SUB	Substrate (tetramethylbenzidin), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl single-use 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
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure 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.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- **Preparation of the extraction buffer:** The **extraction buffer concentrate (EXBUF)** has to be diluted with ultrapure water **1:2.5** before use (90 ml EXBUF + 135 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at 37 °C in a water bath. The **EXBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Extraction buffer** (1:2.5 diluted EXBUF) can be stored in a closed flask at **2–8 °C for 3 months**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C for 4 weeks.**
- **Preparation of the detection antibody:** The **lyophilised detection antibody concentrate (AB)** is stable at **2–8 °C** until the expiry date stated on the label. Before use, the AB has to be reconstituted with **250 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. The **detection antibody concentrate** (reconstituted AB) can be stored at **-20 °C for 4 weeks**. The detection antibody concentrate is further diluted **1:101** in wash buffer (100 µl detection antibody concentrate + 10 ml wash buffer). **The detection antibody** (1:101 diluted detection antibody concentrate) **is not stable and cannot be stored.**

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The **CONJ** is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **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) 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 utilises 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 the microtiter wells coated with high affinity anti-S100A8/S100A9 antibodies. During the first incubation step, S100A8/S100A9 in the samples is bound by the immobilised 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 the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

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 **10–20 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.

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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 (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) 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

Analytical sensitivity see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG 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 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 should 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 analysed with each run.
- Reagents should not be used beyond the expiration date stated 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.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for 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

1. Hildebrand, F. et al., 2013. Inflammation-associated enterotypes, host genotype, cage and inter-individual effects drive gut microbiota variation in common laboratory mice. *Genome biology*, **14**(1), p.R4.
2. Nelson, D. a et al., 2012. An expanded myeloid derived suppressor cell population does not play a role in gammaherpesvirus-exacerbated breast cancer metastases. *Infectious agents and cancer*, **7**(1), p.22.

3. Prinzen, C. et al., 2009. Differential gene expression in ADAM10 and mutant ADAM10 transgenic mice. *BMC genomics*, **10**, p.66.
4. Soro-Paavonen, A. et al., 2008. Receptor for advanced glycation end products (RAGE) deficiency attenuates the development of atherosclerosis in diabetes. *Diabetes*, **57**(9), pp.2461–2469.
5. Volz, H.C. et al., 2012. S100A8/A9 aggravates post-ischemic heart failure through activation of RAGE-dependent NF-κB signaling. *Basic research in cardiology,* **107**(2), p.250.
6. Wiechert, L. et al., 2012. Hepatocyte-specific S100a8 and S100a9 transgene expression in mice causes Cxcl1 induction and systemic neutrophil enrichment. *Cell communication and signaling : CCS*, **10**(1), p.40.
7. Yamamoto, Y. et al., 2011. Septic shock is associated with receptor for advanced glycation end products ligation of LPS. *Journal of immunology* (Baltimore, Md. : 1950), **186**(5), pp.3248–57.

Used symbols:

 Temperature limitation

Catalogue Number

For research use only

To be used with

 Manufacturer

Contains sufficient for <n> tests

Lot number

 Use by

 Attention

 Consult instructions for use

Consult specification data sheet