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User's Manual

Mouse/Rat S100A8/S100A9 ELISA Kit

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Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

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.

General Description

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-indiumlabelled granulocytes is very costly (patient's hospitalisation, 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.

Principles of Testing

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 labelled antimouse 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.

Reagents And Materials Provided

1. PLATE Microtiter plate, pre-coated 12 × 8 wells

2. WASHBUF Wash buffer concentrate, 10x, 2 × 100 ml
3. EXBUF Extraction buffer concentrate, 2.5×, 90 ml
4. Detection antibody concentrate,(monoclonal anti-S100A8/S100A9 antibody), lyophilised 250 µl
5. STD S100A8/S100A9 standards, lyophilised (0; 0.25; 0.98; 3.9; 15.6 ng/ml) 2 × 5 vials
6. CTRL1 Controls, lyophilised (see specification for range) 2 × 1 vial
7. CTRL2 Controls, lyophilised (see specification for range) 2 × 1 vial
8. CONJ Conjugate concentrate (anti-mouse, peroxidase labelled), 200 µl
9. SUB Substrate (tetramethylbenzidin), ready-to-use 1 × 15 ml
10. STOP Stop solution, ready-to-use 1 × 15 ml

Materials Required But Not Supplied

1. Ultrapure water* (* We recommend 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).
2. Laboratory balance
3. Calibrated precision pipettors and 10–1000 µl single-use tips
4. Foil to cover the microtiter plate
5. Horizontal microtiter plate shaker with 37 °C incubator
6. Multi-channel pipets or repeater pipets
7. Centrifuge, 3000 g
8. Vortex
9. Standard single-use laboratory glass or plastic vials, cups, etc., e.g. reaction vessels 1.5 ml or 15 ml
10. Microtiter plate reader

Storage

Store all contents at 2-8°C.

Specimen Collection And Preparation

1. Faeces

Each sample must be extracted **1:50** in extraction buffer. E. g.:

100 mg faeces + 5 ml extraction buffer in a standard laboratory 15 ml reaction vessel

or

20 mg faeces + 1 ml extraction buffer in a standard laboratory 1.5 ml reaction vessel.

a) The sample has to be thawed. Weigh the sample, place in the reaction vessel, pipette in the extraction buffer and close tightly. Soak for approx. 10 minutes. **Important:** Allow the extraction buffer to reach room temperature.

b) Vortex the reaction vessel until a homogeneous suspension is formed. For particularly solid faecal samples, we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device. Floating material like shells of grains can be neglected.

c) Centrifuge the supension at 3000 x g for 10 minutes. A small portion of the suspended sample can also be transferred to a 1.5 ml or 2.0 ml reaction vessel for this purpose.

d) The supernatant is transferred to a fresh reaction vessel. Make sure that the sediment will not be dispersed again.

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

2. EDTA-Plasma/Serum

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

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

3. Urine

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

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

4. Cell culture supernatants

Samples should be diluted at least 1:2 with wash buffer before assaying. The medium is determined as a blank in the same dilution as the cell culture supernatants.

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

Reagent Preparation

1. To run the assay more than once, ensure that reagents are stored at the condi-tionsstated 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.
2. Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
3. **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water 1:10 before use (e. g. 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** can be used until the expiry date stated on the label when stored at **2–8 °C. Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C** for 1 month.
4. **Preparation of the extraction buffer:** The **extraction buffer concentrate (EXBUF)** has to be diluted with ultrapure water **1:2.5** before use (e. g. 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** can be used until the expiry date stated on the label when stored at **2–8 °C. Extraction buffer** (1:2.5 diluted EXBUF) can be stored in a closed flask at **2–8 °C for 3 months**.
5. The **lyophilised standards (STD)** and **controls (CTRL)** can be used until the expiry date stated on the label when stored at 2–8 °C. 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 -20 °C for 4 weeks.**

6. **Preparation of the detection antibody:** The **lyophilised detection antibody concentrate (AB)** can be used until the expiry date stated on the label when stored at **2–8 °C**. 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 (e. g. 100 µl detection antibody concentrate + 10 ml wash buffer). The detection antibody (1:101 diluted detection anti-body concentrate) **is not stable and cannot be stored**.
7. **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (e. g. 100 µl CONJ + 10 ml wash buffer). The **CONJ** can be used until the expiry date stated on the label when stored at **2–8 °C. Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored**.
8. All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8 °C**.

Assay 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 accord-ingto the specific features of the respective automated platform.

We recommend to carry out the tests in duplicate.

1. Before use, wash the wells 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2. Add each 100 µl standards/controls/prepared samples into the respective wells. Only if cell culture supernatants are used as sample material, pipette 100 µl of medium into the respective wells as a blank.
3. Cover the strips and incubate for 1 hour at 37 °C on a horizontal shaker.*
4. Discard the content of each well and wash 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5. Add 100 µl detection antibody (diluted AB) into each well.
6. Cover the strips and incubate for 1 hour at 37 °C on a horizontal shaker.*
7. Discard the content of each well and wash 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8. Add 100 µl conjugate (diluted CONJ) into each well.
9. Cover the strips and incubate for 1 hour at 37 °C on a horizontal shaker.*
10. Discard the content of each well and wash 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11. Add 100 µl substrate (SUB) into each well.
12. Incubate for 10–20 min** at room temperature (15–30 °C) in the dark.
13. Add 100 µl stop solution (STOP) into each well and mix well.
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 incubation steps mentioned above at 550 rpm with an orbit of 2 mm and 37 °C are recommended by the manufacturerer. If there is no possibility to shake at 37 °C, we recommend incubation at 37 °C without shaking.

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

Quality Control

We recommend 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.

Calculation

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.

4. Faeces

The obtained results have to be multiplied by the dilution factor 50 to get the actual concentrations.

5. EDTA-Plasma/Serum

The obtained results have to be multiplied by the dilution factor 100 to get the actual concentrations.

6. Urine

The obtained results have to be multiplied by the dilution factor 3 to get the actual concentrations.

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

7. Cell culture supernatants

The obtained results have to be multiplied by the dilution factor 2 or thedilution factor used to get the actual

concentrations. The concentration of the medium calculated with the dilution factor used must be subtracted from the concentrations of the cell culture supernatants multiplied by the dilution factor used, to obtain the actual concentrations of the cell culture supernatants.

Example

Measured concentration of cell culture supernatant = $conc_{cell\ culture}$ = 3.0 ng/ml

Dilution factor of cell culture supernatant = $df_{cell\ culture}$ = 2

Dilution factor of Medium = $conc_{medium}$ = 0.5 ng/ml

Measured concentration of medium = df_{medium} = 2

Actual concentration of the cell culture supernatant =

$conc_{cell\ culture} \times df_{cell\ culture} - conc_{medium} \times df_{medium}$ = 3.0 ng/ml × 2 – 0.5 ng/ml × 2 = 5 ng/ml

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.

Specificity

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

Precautions

1. All reagents in the kit package are for research use only.
2. Human materials used in kit components were tested and found to benegative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
3. Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzy-maticcolour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided.
4. The 10× Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact. Warning: Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.
5. 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.

TECHNICAL HINTS

1. 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.
2. Control samples should be analysed with each run.

3. Reagents should not be used beyond the expiration date stated on the kit label.
4. Substrate solution should remain colourless until use.
5. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
6. Avoid foaming when mixing reagents.
7. Do not mix plugs and caps from different reagents.
8. The assay should always be performed according to the enclosed manual.

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