Creative Diagnostics®

Version 08-01/25

User's Manual

S100A8/A9 (MRP8/14, Calprotectin) ELISA Kit

REF **DEIA063J**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This assay is an enzyme immunoassay intended for the quantitative determination of calprotectin (MRP 8/14, S100A8/A9) in serum and plasma. For research use only.

General Description

Calprotectin is a calcium-binding protein secreted predominantly by neutrophils and monocytes. The heterocomplex consists of the two proteins, S100A8 (calgranulin A) and S100A9 (calgranulin B), also designated as MRP8 and MRP14, respectively. Expression of S100A8 and S100A9 in epithelial tissues was first described in context with squamous epithelia and with murine and human wound repair. More recently, an association of S100 protein expression with adenocarcinomas in humans has emerged. The genes S100A8 and S100A9 are located in a gene cluster on chromosome 1q21, a region in which several rearrangements that occur during tumor devel- opment have been observed.

Principles of Testing

This ELISA is designed for the quantitative determination of calprotectin (MRP (8/14, S100A8/A9). The assay utilises 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 immobilised antibody. Then a peroxidase labelled 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 colour changes from blue to yellow. The intensity of the yellow colour is directly pro- portional to the calprotectin concentration of sample.

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from standard. Calprotectin, present in the samples, is determined directly from this curve.

Reagents And Materials Provided

1. PLATE Microtiter plate, pre-coated, 12 x 8 wells
2. WASHBUF Wash buffer concentrate, 10x, 2 x 100 ml
3. SAMPLEBUF Sample dilution buffer, ready-to-use, 1 x 100 ml
4. STD Calprotectin standards, ready-to-use, (0; 3,9; 15,6; 62,5; 250 ng/ml), 1 x 5 vials
5. CTRL 1 Control, ready-to-use, (see specification for range), 1 x 1 vial
6. CTRL 2 Control, ready-to-use, (see specification for range), 1 x 1 vial
7. CONJ Conjugate, ready-to-use, 1 x 15 ml
8. SUB Substrate (tetramethylbenzidine), ready-to-use, 1 x 15 ml

9. STOP Stop solution, ready-to-use, 1 x 15 ml

Materials Required But Not Supplied

1. Ultrapure water
2. Calibrated precision pipettors and 101 000 µl single-use tips
3. Foil to cover the microtiter plate
4. Multi-channel pipets or repeater pipets
5. Vortex
6. Standard single-use laboratory glass or plastic vials, cups, etc.
7. Microtiter plate reader

Storage

2-8°C

Specimen Collection And Preparation

Sample stability and storage

Calprotectin is stable in serum for 7 days at 2–8°C as well as for 3 days at room temperature. At -20°C, the samples can be stored for up to 6 months. More than 3 freeze thaw cycles are to be avoided.

Calprotectin is not stable in plasma.

Preanalytic handling

Significant differences in the calprotectin levels can be observed due to different sample preparation procedures, e. g. up to 10-fold higher serum levels compared to the plasma calprotectin concentrations. The reasons are as follows:

Granulocytes are activated during serum clotting and release granulocyte-activating markers. The time between serum collecting and analysis as well as repeated freeze-thaw cycles don't cause a calprotectin concentration shift.

On the contrary, in the case of plasma samples, varying the time between sampling and analysis or the number of freeze-thaw cycles will cause variation in the observed calprotectin levels. Therefore, the preanalytical conditions of plasma samples should beheld constant. This is a general requirement independent of the used test-system. We recommend the use of serum samples for calprotectin determinations.

Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.

Serum samples

Serum samples must be diluted 1:100 with sample dilution buffer (SAMPLEBUF) before performing the assay, e. g.

50 µl sample + 450 µl SAMPLEBUF = dilution I (1:10)

50 µl dilution I + 450 µl SAMPLEBUF = dilution II (1:10) Final dilution 1:100

For analysis, pipet 100 µl of dilution II per well.

Plasma samples

EDTA plasma samples must be diluted 1:30 with sample dilution buffer (SAMPLEBUF) before performing the assay, e. g.

20 µl sample + 580 µl (SAMPLEBUF).

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

Reconstitution And Storage

1. To run the assay more than once, ensure that reagents are stored at the condi- tions 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.
2. 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 con- centration in the concentrate. Before dilution, the crystals have to be redis- solved 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.
3. All other test reagents are ready-to-use. Test reagents are stable until the ex- piry 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. Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8°C. Strips are stable until expiry date stated on the label.

For automated ELISA processors the given protocol may need to be adjusted accord- ing to the specific features of the respective automated platform.

We recommend to carry out the tests in duplicate.

1. Add each 100 µl standards/controls/diluted samples into the respec- tive wells.
2. Cover the strips and incubate for 30 min at room temperature (15–30°C).
3. 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.
4. Add 100 µl conjugate (CONJ) into each well.
5. Cover the strips and incubate for 30 min at room temperature (15–30°C).
6. 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.
7. Add 100 µl substrate (SUB) into each well.
8. Incubate for 10–20 minutes* at room temperature (15–30°C) in the dark.
9. Add 100 µlstop solution (STOP) into each well and mix well.

10. 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 colour change and to stop 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 logarith- mic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a valueless 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 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.

Serum

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

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

EDTA plasma

The obtained results have to be multiplied by the dilution factor of 30 to get the actual concentrations.

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

Sensitivity

Limit of blank, LoB 0.522 ng/ml

Limit of detection, LoD 0.789 ng/ml

Limit of quantitation, LoQ 0.897 ng/ml

Specificity

Human

Precautions

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

• Human materials used in kit components were tested and found to be nega- tive 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 or ProClin are hazardous to health and the environment. Substrates for enzy- matic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided.

• The 10x 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 wa- ter for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.

• 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 out immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

• Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not to assemble 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 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.

Limitations

Samples with concentrations above the measurement range 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:

LoQ × sample dilution factor to be used

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