

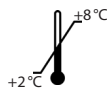
IDK[®] Calprotectin ELISA

*For the in vitro determination of
calprotectin (MRP 8/14, S100A8/A9) in stool*

Valid from 2019-01-01

REF KR6927

Σ 96



RUO

REF KR6927.20

Σ 20 x 96

+2°C



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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of calprotectin (MRP (8/14, S100A8/A9) in stool. For research use only. Not for diagnostic procedures.

2. INTRODUCTION

Alternative names:

- Calgranulin A: MRP8, S100A8, CP-10 (in mouse)
- Calgranulin B: MRP14, S100A9,
- MRP8/14: L1, (p8,14), p34

Calprotectin is a calcium-binding protein secreted predominantly by neutrophils and monocytes. Faecal calprotectin is a marker for neoplastic and inflammatory gastrointestinal diseases.

3. MATERIAL SUPPLIED

| Cat. No. | Label | Kit components | Quantity for cat. no. | |
|--------------|-----------|--|-----------------------|--------------------|
| | | | KR6927 | KR6927.20 |
| KR6927 | PLATE | Microtiter plate, pre-coated | 12x 8 wells | 20x 12x 8 wells |
| KR0001.C.100 | WASHBUF | Wash buffer concentrate, 10x | 2x 100 ml | 40x 100 ml |
| KR6927 | CONJ | Conjugate, ready-to-use peroxidase-labelled | 1 x 15 ml | 20x 15 ml |
| KR6927 | STD | Standards, ready to use (0; 13; 52; 210; 840 ng/ml) | 1 x 5 vials | 10x 5 vials |
| KR6927 | CTRL1 | Control, ready-to-use (see specification for range) | 1 x 1 vial | 10x 1 vial |
| KR6927 | CTRL2 | Control, ready-to-use (see specification for range) | 1 x 1 vial | 10x 1 vial |
| KR6927 | SAMPLEBUF | Sample dilution buffer, ready-to-use | 1 x 100 ml | 10x 100 ml |
| KR0002.15 | SUB | Substrate (tetramethylbenzidine), ready-to-use | 1 x 15 ml | 20x 15 ml |
| KR0003.15 | STOP | Stop solution, ready-to-use | 1 x 15 ml | 20x 15 ml |

| Cat. No. | Label | Kit components | Quantity for cat. no. | |
|--------------|--------------|---|-----------------------|-------------|
| | | | KR6927 | KR6927.20 |
| KR6999.C.100 | IDK Extract® | Extraction buffer concentrate <i>IDK Extract® 2.5x</i> | 1 x 100 ml | 10 x 100 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*
- Stool sample application system such as Cat. No.: K 6998SAS
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Multi-channel pipets or repeater pipets
- 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 assay more than once, ensure that reagents are stored at 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.
- **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 *IDK Extract®*** has to be diluted with ultrapure water **1:2.5** before use (100 ml *IDK Extract®* + 150 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 *IDK Extract®* is stable at **2–8 °C**

until the expiry date stated on the label. Extraction buffer (1:2.5 diluted *IDK Extract*®) can be stored in a closed flask at **2–8 °C for 4 months**.

- 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. STORAGE AND PREPARATION OF SAMPLES

Stability and storage of samples

Raw stool

Calprotectin in stool is described to be stable for at least 3 days at room temperature (Tøn et al. (2000) Clin Chim Acta). Nevertheless, we recommend storing the samples for no more than 48 h at 2–8 °C and transporting the samples at room temperature for maximum 2 days. Long term storage up to 12 months is recommended at -20 °C. Allow frozen samples to thaw slowly, preferably at 2–8 °C, and warm the samples to room temperature before analysis. Avoid repeated freezing and thawing of the sample. Freezing can cause neutrophil granulocytes in the stool sample to burst and release calprotectin. Therefore frozen samples can be expected to contain slightly elevated concentrations of calprotectin compared to fresh samples.

Chemical or biological additives in stool sample tubes may interfere with *IDK*® Calprotectin. Therefore use only empty tubes or tubes filled with the extraction buffer *IDK Extract*® supplied by Immundiagnostik AG.

Stool extracts

Stool extract is stable for nine days at room temperature, 2–8 °C or -20 °C. Avoid more than three freeze-thaw cycles.

Extraction of the stool samples

Extraction buffer (1:2.5 diluted *IDK Extract*®) is used as a sample extraction buffer. We recommend the following sample preparation:

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

Stool sample tube – Instructions for use

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

SAS with 1.5 ml sample extraction buffer:

Applied amount of stool: 15 mg

Buffer Volume: 1.5 ml

Dilution Factor: 1:100

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 particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.
- b) Fill the **empty stool sample tube** with **1.5 ml extraction buffer** (1:2.5 diluted *IDK Extract*®) before using it with the sample. **Important:** Allow the sample extraction buffer to reach room temperature.
- c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has 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, leaving 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 sample extraction buffer for ~ 10 minutes improves the result.
- e) Allow the sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
- f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:100

Dilution of samples

The supernatant of the sample preparation procedure (dilution I) is diluted **1:25 in sample dilution buffer (SAMPLEBUF)**. For example:

40 µl supernatant (dilution I) + **960 µl** sample dilution buffer, mix well = **1:25 (dilution II)**. This results in a final dilution of 1:2500.

100 µl of **dilution II** are used in the test.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of calprotectin.

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 proportional 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 the standard. Calprotectin, 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.

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 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. | Add each 100 µl standards/controls/diluted samples into the respective 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 min* at room temperature (15–30 °C) in the dark . |
| 9. | Add 100 µl stop 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 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.

Stool samples

The obtained results have to be multiplied by the **dilution factor** of **2 500** (dilution I × dilution II) to get the actual concentrations.

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

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this greater 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:

LoB × sample dilution factor to be used

LoB 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.

Reference range

1 g stool is equivalent to 1 ml.

We recommend each laboratory to establish its own reference concentration range.

11. PERFORMANCE CHARACTERISTICS

The following values have been estimated based on the concentrations of the standard curve without considering possibly used sample dilution factors.

Accuracy – Precision

Repeatability (Intra-Assay); n=20

The repeatability was assessed with 2 stool samples under **constant** parameters (same operator, measurement system, day and kit lot).

| Sample | Mean value [ng/ml] | CV [%] |
|--------|--------------------|--------|
| 1 | 89.19 | 5.6 |
| 2 | 229.05 | 3.2 |

Reproducibility (Inter-Assay); n=25

The reproducibility was assessed with 3 stool samples under **varying** parameters (different operators, measurement systems, days and kit lots).

| Sample | Mean value [ng/ml] | CV [%] |
|--------|--------------------|--------|
| 1 | 59.17 | 11.60 |
| 2 | 253.34 | 8.82 |
| 3 | 490.31 | 9.11 |

Analytical sensitivity

Limit of blank, LoB

0.957 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 to assemble wells of different microtiter plates for analysis, even if they are of the same batch as wells from already opened microtiter plates are exposed to different conditions as sealed ones.
- 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.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- *IDK®* and *IDK Extract®* are trademarks of Immundiagnostik AG.
- 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

General literature












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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 | | |