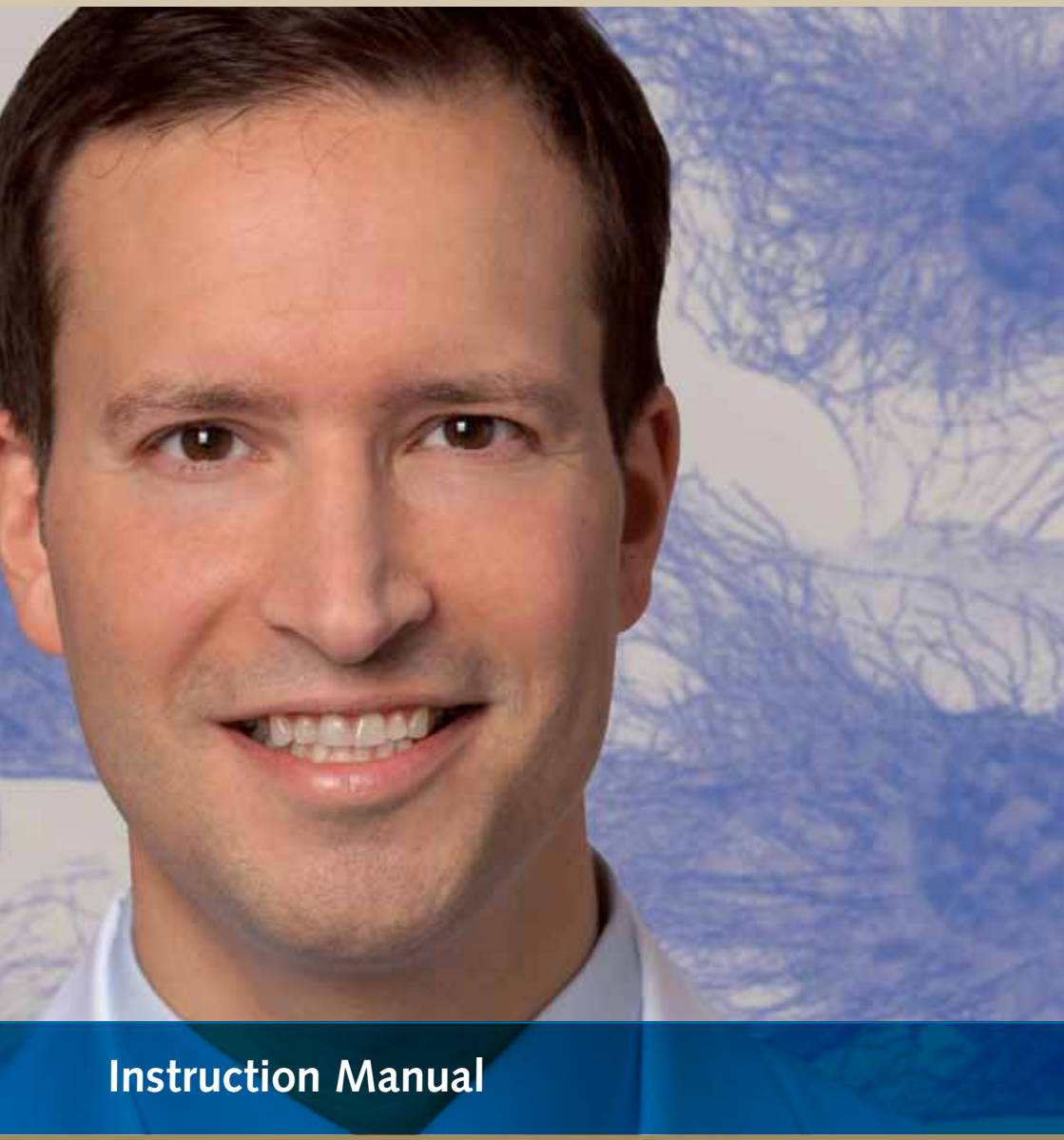
Human Calprotectin (MRP 8/14) ELISA Kit



Instruction Manual

PromoKine

Contents

Intended Use

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for quantitative determination of Calprotectin (MRP8/14) in serum and plasma. It is for research use only.

Introduction

- Alternative names of calprotectin:

  MRP8/14, L1, (p8,14), p34
- Alternative names of the two proteins forming the heterocomplex calprotectin:

  S100A8, Calgranulin A, MRP8 (Migration inhibition factor-related protein-8), CP-10 (in mouse)

  S100A9, Calgranulin B, MRP14 (Migration inhibition factor-related protein-14)

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 development have been observed.

Elevated MRP8/14 levels have been found in many sites of inflammation and in the extracellular fluid of patients with many types of inflammatory conditions. The concentration of MRP8/14 in blood is increased in patients with rheumatoid arthritis, cystic fibrosis, multiple scerlosis, and HIV infections, while elevated MRP8/14 levels have been detected in stool of patients with Crohn's disease and colorectal cancer [1-5]. Extracellular MRP8/14 has antimicrobial, antigrowth and apoptotic effects. It suppresses the growth of some fungi and bacteria [1,2].

It also suppresses the proliferation of several different types of cells including: macrophages, lymphocytes, hematopoietic progenitors, and tumor cell lines. MRP8/14 can also induce apoptosis of some tumor cell lines [1,2].

Hermani et al. (2005) [6] reported recently that enhanced expression of S100A8 and S100A9 is an early event in prostate tumor genesis and may contribute to development and progression or extension of prostate carcinomas. Furthermore, they tested the value of S100A9 as a serum marker for prostate cancer comparing the serum concentrations of S100A9 in cancer patients with healthy controls or patients with benign prostatic hyperplasia (BPH). Significantly increased S100A9 serum levels in prostate cancer were found in prostate cancer patients compared to patients with BPH, the latter exhibiting values similar to that obtained for healthy individuals.

Pathological significance and clinical application

The diagnostic value and advantage of MRP8/14 over other disease markers is that they are preformed and released immediately upon activation of the respective cell population. Other markers may be generated in downstream events or need to be synthesized *de novo* in the liver. Various conditions have shown significant correlation of MRP8/14 (or MRP8, MRP14) levels with disease activity:

- Concentrations of MRP8/14 in serum, and particularly in synovial fluid, correlate strongly with disease activity in **rheumatoid arthritis**.
- Plasma MRP8/14 levels are very early, specific and sensitive prediction markers for acute **rejection in kidney allograft transplantation**.
- Serum MRP8/14 concentration is a prognostic marker of recurrent **infection** and of poor survival in **alcoholic liver cirrhosis**.
- MRP8/14 is useful for evaluating the extent of **periodontal inflammation**.
- In **cerebral malaria**, MRP 8/14 expression correlates with microglial activation in brain.
- MRP8/14 is present in **urinary stones** and in **dental calculus**.
- S100A9 in serum may serve as a useful marker for **discrimination** between **prostate cancer** and **benign prostatic hyperplasia** (BPH).

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
STD	Calprotectin standards, lyophilized (0; 3.9; 15.6; 62.5; 250 ng/ml)	2 x 5 vials
CTRL 1	Control, lyophilized (see specification for range)	2 x 1 vial
CTRL 2	Control, lyophilized (see specification for range)	2 x 1 vial
CONJ	Conjugate, ready-to-use	15 ml
SUB	TMB substrate (Tetramethylbenzidine), ready to use	15 ml
STOP	ELISA stop solution, ready to use	15 ml

Material and Equipment Required but not Provided

- Ultra-pure water*
- Laboratory balance
- Precision pipettors calibrated and tips to deliver 10-1000 µl
- Covering foil for the microtiter plate
- Horizontal microtiter plate shaker with 37°C incubator
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 or 405 nm (reference wave length 620 or 690 nm)

* We recommend 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).

Preparation and Storage of Reagents

- To run assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. 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 with aqua bidest. **1:10** before use (100 ml concentrate + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C using a water bath 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** can be stored in a closed flask at **2-8°C for one month**.
- The lyophilized **STD** (standards) and **CTRL** (control) are stable at **2-8°C** until the expiry date stated on the label. The **STD** (standards) and **CTRL** (control) must be reconstituted with **500 µl** aqua bidest. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted standards and control can be stored at 2-8°C for four weeks.**
- All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

Sample Preparation

Serum and Plasma samples

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 granulocyteactivating 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 be held constant. This is a general requirement independent of the used test-system.

We recommend the use of serum samples for calprotectin determinations.

Serum samples should be diluted **1:100** with **sample dilution buffer** 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)

EDTA Plasma samples should be diluted **1:30** with **sample dilution buffer** before performing the assay.

Assay Procedure

Principle of the test

The assay utilizes the two-site "sandwich" technique with two selected monoclonal antibodies that bind to human Calprotectin. Standards, controls and prediluted patient 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 immobilized antibody.

In a next incubation step, a biotinylated monoclonal anti-human Calprotectin antibody is added to each microtiter well. Then a peroxidase labeled exravidin conjugate is added to each well and the following complex is formed: capture antibody - human Calprotectin – biotinylated detection antibody - Peroxidase conjugate. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color 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 standard. Calprotectin present in the patient samples, is determined directly from this curve.

Test Procedure

1. Bring all reagents and samples to room temperature (15-30°C) and mix well.
2. Mark the positions of STD /SAMPLE/CTRL (Standards/Sample/Control) in duplicate on a protocol sheet.
3. 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.
4. Note: 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.
5. We recommend to carry out the tests in duplicate.
6. Add **100 µl** of **STD/SAMPLE/CTRL** (Standard/Sample/Control) in duplicate into respective well.
7. Cover plate tightly and incubate for **30 minutes at room temperature**.
8. Aspirate the contents of each well. Wash 5 times by dispending **250 µl** of **diluted WASHBUF** (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
9. Add **100 µl CONJ** (conjugate) into each well.

10. Cover plate tightly and incubate for **30 minutes at room temperature**.
11. Aspirate the contents of each well. Wash 5 times by dispending **250 µl** of **diluted WASHBUF** (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
12. Add **100 µl of SUB** (substrate) into each well
13. Incubate for 10 - 20 minutes at room temperature (15-30°C) in the dark*.
14. Add **100 µl** of **STOP** (stop solution) into each well, mix thoroughly
15. 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 color change is temperature sensitive. We recommend to observe the procedure of the color change and to stop the reaction upon good differentiation.

Results

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm

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

2. Point-to-point-calculation

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

3. Spline-algorithm

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001). 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

For calculation of calprotectin concentration in serum, the result must be multiplied by the dilution factor of **100**.

EDTA Plasma

For calculation of calprotectin concentration in plasma, the result must be multiplied by **dilution factor of 30**.

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

Limitations

Samples with Calprotectin concentrations above the measurement range (see definition below) must be further diluted and re-assayed.

Samples with Calprotectin 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 x sample dilution factor to be used

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

LoB x sample dilution factor to be used*

**Limit of Blank*

Quality Control

PromoCell recommends the use of external/commercial control samples for internal quality control, if available.

Control samples should be analyzed 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.

Expected values

Reference range

Calprotectin in serum of healthy persons: < 3 µg/ml (< 3000 ng/ml) We recommend each laboratory to establish its own reference concentration range.

Performance Characteristics

Precision and reproducibility

Intra-Assay (n=80)

Sample	Calprotectin [ng/ml]	VK [%]
1	447.7	3.7
2	784.6	7.9

Inter-Assay (n=10)

Sample	Calprotectin [ng/ml]	VK [%]
1	485.28	5.5
2	816.11	7.0

Specificity

No cross reactivity with MPR 8/14 in mouse serum was observed.
No cross reactivity was observed with the following plasma proteins:

Lysozyme – 0%

PMN-Elastase – 0%

Myeloperoxidase – 0%

Lactoferrin – 0%

Analytical Sensitivity

Limit of Blank, LoB	0.52 ng/ml
Limit of Detection, LoD	0.78 ng/ml
Limit of Quantitation, LoQ	0.78 ng/ml

Spiking Recovery

Two samples were spiked with different Calprotectin standards and measured using this assay (n=2).

Sample	Sample unspiked [ng/ml]	Spike [ng/ml]	Calprotectin expected [ng/ml]	Calprotectin measured [ng/ml]
Sample 1	4.29	8.36	12.65	11.39
	4.25	13.19	17.44	16.41
	4.21	16.83	21.04	19.32
	4.13	24.12	28.25	27.21
Sample 2	7.35	8.36	15.71	15.42
	7.28	13.19	20.47	20.27
	7.21	16.83	24.04	23.98
	7.08	24.12	31.20	30.27

Dilution Recovery

Two serum samples were diluted and analysed. The results are shown below (n=2).

Sample	Dilution	Calprotectin expected [ng/ml]	Calprotectin measured [ng/ml]
Sample A	1:50		9.34
	1:100	4.67	4.53
	1:200	2.34	2.27
	1:400	1.17	1.13

Sample	Dilution	Calprotectin expected [ng/ml]	Calprotectin measured [ng/ml]
Sample B	1:50		18.17
	1:100	9.09	8.9
	1:200	4.54	4.25
	1:400	2.27	2.11

Precautions

- For *in vitro* research use only.
- Quality control guidelines should be observed.
- 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 color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

Technical Hints

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiration date shown 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.
- The assay should always be performed according the enclosed manual.

General Notes on the Test and Test Procedure

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* research use only.
- Guidelines for medical laboratories should be observed.
- 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 supplier, may influence the results of the test. PromoCell can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to PromoCell together with a written complaint.

References

- Striz I, Trebichavsky I: Calprotectin - a pleiotropic molecule in acute and chronic inflammation. *Physiol Res* 2004, 53: 245-253.
- Yui S, Nakatani Y, Mikami M: Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. *Biol Pharm Bull* 2003, 26: 753-760.
- Odink K, Cerletti N, Bruggen J, Clerc RG, Tarcsay L, Zwadlo G *et al*.: Two calciumbinding proteins in infiltrate macrophages of rheumatoid arthritis. *Nature* 1987, 330: 80-82.
- Wilkinson MM, Busuttil A, Hayward C, Brock DJ, Dorin JR, Van H, V: Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues. *J Cell Sci* 1988, 91 (Pt 2): 221-230.
- Muller F, Froland SS, Aukrust P, Fagerhol MK: Elevated serum calprotectin levels in HIV-infected patients: the calprotectin response during ZDV treatment is associated with clinical events. *J Acquir Immune Defic Syndr* 1994, 7: 931-939.
- Hermani A et al. Calcium-Binding Proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer. *Clin Cancer Res* 2005, 11: 5146- 5152.

Ordering Information

Product Name	Product Description	Size	Catalog Number
Calprotectin (MRP 8/14) ELISA Kit, human	Human Calprotectin (MRP 8/14) ELISA Kit	96 Tests	PK-EL-K6935

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

Email: info@promokine.info