Human Calprotectin ELISA Kit

Enzyme Immunoassay for the quantification of Calprotectin in human serum, plasma, milk, urine, saliva, CSF, cell lysate and cell culture supernatants samples

Catalog number: ARG82070

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Calprotectin is a complex of the mammalian proteins S100A8 and S100A9. In the presence of calcium, calprotectin is capable of sequestering the transition metals iron, manganese and zinc via chelation. This metal sequestration affords the complex antimicrobial properties. Calprotectin is the only known antimicrobial manganese sequestration protein complex. Calprotectin comprises as much as 60% of the soluble protein content of the cytosol of a neutrophil, and it is secreted by an unknown mechanism during inflammation. Faecal calprotectin has been used to detect intestinal inflammation (colitis or enteritis) and can serve as a biomarker for inflammatory bowel diseases and rheumatoid arthritis. Other names for calprotectin include MRP8-MRP14, calgranulin A and B, cystic fibrosis antigen, L1, 60BB antigen, and 27E10 antigen.

Calprotectin constitutes up to 60% of soluble protein content in the cytosol of neutrophil granulocytes, and it can be found at a lower concentration in monocytes, macrophages, and squamous epithelial cells. Calprotectin enters into pus and abscess fluid during neutrophil cell death, along with other antimicrobial proteins.

Mammalian cells secrete calprotectin during the inflammatory response. Plasma calprotectin is elevated in persons with metabolic syndrome, a disease characterized by chronic inflammation. Calprotectin is secreted in the mouth during inflammation of the gingiva and during oral candidiasis infection. People who have mutations in the calprotectin gene appear susceptible to serious gum infections. Manganese sequestration by calprotectin is likely important during lung inflammation. The exact mechanism by which S100A8 and S100A9 is secreted by mammalian cells during inflammation remains unknown. [provided by Wikipedia: Calprotectin]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Calprotectin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Calprotectin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Calprotectin is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Calprotectin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Calprotectin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the kit as Storage information below. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated Microtiter Plate	12 x 8 wells	4°C
Human Calprotectin Standard (4.5 ng)	1 vial (Lyophilized)	4°C (store at -20°C after reconstitution)
25X Biotinylated Human Calprotectin antibody concentrate	200 µl	-20°C
100X Streptavidin-HRP concentrate	80 µl	-20°C
10X Diluent concentrate	30 ml	4°C
20X wash buffer concentrate	2 X 30 ml	4°C
TMB Substrate	7 ml (Ready to Use)	4°C
STOP solution	11 ml (Ready to Use)	4°C
Plate sealer	3 pieces	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the components at recommended temperature.
- Store 25X Biotinylated Human Calprotectin antibody concentrate and 100X Streptavidin-HRP concentrate at-20°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at-20°C after reconstituting with Diluent.
- Briefly spin down the standards and solutions before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- If crystals are observed in the 20X Wash buffer or 10X Diluent, warm to RT and mix gently until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before opening and using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.

- Change pipette tips between the addition of different reagent or samples.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines.

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect supernatants. Dilute samples 2,000X into 1X Diluent buffer and assay (Dilution factor=2000). The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and collect serum. Dilute samples 2,000X into 1X Diluent buffer and assay (Dilution factor=2000). The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants: Collect cell culture media and centrifuge at 1500 rpm for 10 minutes at 4°C to remove debris. Collect supernatants and assay. The undiluted samples can be aliquoted and stored at-80°C. Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample tube. Centrifuge samples at 800 x g for 10 minutes. It is suggested dilute samples 200X with 1X Diluent buffer and assay

immediately (Dilution factor=200. User should determine optimal dilution factor depending on application needs, usually dilute samples within 20X-2000X). The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. It is suggested dilute samples 10,000X with 1X Diluent buffer and assay immediately (Dilution factor=10000. User should determine optimal dilution factor depending on application needs, usually dilute samples within 1,000X-100,000X). The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. It is suggested dilute samples 80,000X with 1X Diluent buffer and assay immediately (Dilution factor=80000. User should determine optimal dilution factor depending on application needs, usually dilute samples within 50,000X-250,000X). The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. It is suggested dilute samples 200X with 1X Diluent buffer and assay immediately (Dilution factor=200. User should determine optimal dilution factor depending on application needs, usually dilute samples within 20X-2000X). The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Lysate: Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis

Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 10^6 cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. Samples can be stored at-80°C. Avoid repeated freeze-thaw cycles.

Note:

- Applicable samples may also include other biofluids, and tissue lysates. If necessary, user should determine optimal dilution factor depending on application needs.

- Dilution Note (For duplicate):

a) For 100X dilution: add 5 µL of samples into 495 µL of 1X Diluent buffer, mix well.

b) For 200X dilution: add 5 µL of samples into 995 µL of 1X Diluent buffer, mix well. (For Urine, CSF)

c) For 2,000X dilution: add 10 µL of diluted samples from a) into 190 µL of 1X Diluent buffer, mix well. (For serum, plasma; Step a: 100X & Step c: 20X)

d) For 10,000X dilution: add 5 µL of diluted samples from a) into 495 µL of 1X Diluent buffer, mix well. (For Saliva, Step a: 100X & Step d: 100X)

e) For 80,000X dilution: add 20 µL of diluted samples from d) into 140 µL of 1X Diluent buffer, mix well. (For Milk, Step a: 100X & Step d: 100X & Step e: 8X)

REAGENT PREPARATION

- **1X Diluent buffer**: Dilute **10X** Diluent concentrate into **distilled water** to yield 1X Diluent buffer (E.g. 10 ml of 10X Diluent + 90 ml of distilled water). If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use. The diluted 1X Diluent can be stored for up to 30 days at 2-8°C.
- **Wash buffer**: Dilute **20X** Wash buffer into **distilled water** to yield 1X Wash buffer. (E.g. 10 ml of 20X wash buffer + 180 ml of distilled water) If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use.
- **Biotinylated Human Calprotectin antibody**: Briefly spin down the **25X** Biotinylated Human Calprotectin antibody. Dilute the antibody with **1X Diluent buffer** (E.g. 100 µl of 25X biotin-conjugated antibody + 2400 µl of 1X Diluent buffer). Any remaining undiluted solution should be frozen at -20°C.
- **Streptavidin-HRP conjugate**: Spin down the **100X Streptavidin-HRP Conjugate** briefly and dilute the desired amount of the conjugate with **1X Diluent buffer** (E.g. 40 µl of Streptavidin-HRP conjugate + 3960 µl of 1X Diluent buffer). Any remaining undiluted solution should be frozen at -20°C.
- **Standard peptide**: Reconstitute the Standard vial (4.5 ng) with **0.9 ml** of **1X Diluent buffer.** The concentration of this stock solution is 5 ng/ml. Allow it to sit for 10 minutes at room temperature (20-25°C) with gentle agitation prior to making dilutions. Mix well and spin down before use.

Dilute standard solutions according to the table below and make serial dilutions of 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.313 ng/ml, 0.156 ng/ml and 0.078 ng/ml. And the 1X Diluent buffer serves as the zero standard. Any remaining stock standard solution should be stored at-20°C and used within 30 days. Aliquot to avoid repeated freeze-thaw cycles is recommended.

Dilute Calprotectin standard as according to the table below:

Standard No.	Standard	1X Diluent Buffer	Concentrations (ng/ml)
S1	300 µl	0 µl	5
S2	150 µl of S1	150 µl	2.5
S3	150 µl of S2	150 µl	1.25
S4	150 µl of S3	150 µl	0.625
S5	150 µl of S4	150 µl	0.313
S6	150 µl of S5	150 µl	0.156
S7	150 µl of S6	150 µl	0.078
Blank	0	150 µl	0

ASSAY PROCEDURE

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (RT, 20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. The remaining microplate strips may be stored for up to 30 days in a vacuum desiccator.
3. Standards, samples and controls should be assayed in duplicates.
4. Add **50 µl** of Human Calprotectin **Standard or sample** per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
5. Cover wells with a sealing tape and incubate for **2 hours at RT (20-25°C)**. Start the timer after the last addition.
6. Remove sealer from plate.
7. Aspirate each well and wash, repeating the process 4 times for a total **5 washes** (If a microplate washer is used, wash the wells for a total 6 washes). Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
8. Add **50 µl** of **1X Biotinylated Human Calprotectin antibody** into each well.

Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.

9. Reseal the plate with sealer. Incubate for **1 hour at RT.**
10. **Wash** as according to step 7.
11. Add **50 µl** of **1X Streptavidin-HRP solution** into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
12. Reseal the plate with sealer. Incubate for **30 minutes at RT.** (Turn on the microplate reader and set up the program in advance.)
13. **Wash** as according to step 7.
14. Add **50 µl** of **TMB substrate solution** into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
15. Incubate for **30 minutes at RT** or until the optimal blue color density develops. (**Protect from light**)
16. Add **50 µl** of **STOP solution** into all wells to stop the reaction. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
17. Read the OD with a microplate reader at **450 nm immediately.** If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings. So it is recommended read the absorbance within 10 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

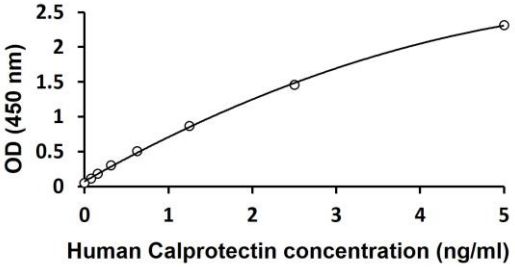
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



Reference Values:

• Normal human calprotectin plasma and serum levels range from 1000 – 4000 ng/ml.

• Plasma and serum samples from healthy adults were tested (n=40). On average, human calprotectin level was 2409 ng/ml.

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of human Calprotectin as calculated by 2SD from the mean of a zero standard was established to be 0.042 ng/ml.

Standard Range

Standard Range: 0.078- 5 ng/ml

Recovery

89-109%

Linearity

Sample Dilution	Plasma	Serum
1000x	97%	92%
2000x	101%	101%
4000x	106%	108%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.2 % and inter-assay precision was 9.9 %.

Cross Reactivity

No significant cross-reactivity observed with CD9, elastase, lactoferrin, lysozyme, myeloperoxidase, S100-A6, S100-A10, S100-A11, S100-A14, S100-A16, and S100-B.

This kit detects no cross-reactivity with the following factor:

Calprotectin (Bovine)

Calprotectin (Canine)

Calprotectin (Monkey)

Calprotectin (Mouse)

Calprotectin (Rabbit)

Calprotectin (Rat)

Calprotectin (Swine)

Note: 10% FBS in culture medium will not affect the assay.