ab320045 – Human Calprotectin SimpleStep ELISA® Kit (S100A8/S100A9)

For the quantitative measurement of Calprotectin in human serum, plasma (citrate), plasma (EDTA), cell culture supernatant, urine, saliva, milk, cell extract, and tissue extract.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab320045

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human Calprotectin Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Calprotectin Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Calprotectin Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent BAR	6 mL	10 x 6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	2 x 50 mL	+4°C
Sample Diluent NS	12 mL*	N/A	+4°C
Wash Buffer PT 10X	2 x 20 mL	2 x 200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	10 x 96 wells	+4°C
Plate Seal	1	10	+4°C

*Sample Diluent NS is provided but not necessary for this product.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Precautions

Calprotectin is abundantly expressed in saliva and requires precautionary measures to prevent contamination of kit reagents while running this assay. To prevent contamination, it is recommended to clean bench surfaces and all pipettes to be used during the experiment with 10% bleach. Use a surgical mask and maintain clean gloves by either using 70% ethanol or by changing them frequently. Do not leave reagents or the plate opened while working or during assay incubation.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent BAR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent BAR. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the Calprotectin standard sample by adding the volume of 1X Wash Buffer PT indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 24 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 330 µL of 1X Wash Buffer PT into tube number 1 and 150 µL of 1X Wash Buffer PT into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	30	330	24,000	2,000
2	Standard#1	150	150	2,000	1,000
3	Standard#2	150	150	1,000	500
4	Standard#3	150	150	500	250
5	Standard#4	150	150	250	125
6	Standard#5	150	150	125	62.5
7	Standard#6	150	150	62.5	31.3
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:64,000 - 1:4,000
Plasma – Citrate	1:64,000 - 1:4,000
Plasma – EDTA	1:16,000 - 1:1,000
Milk	1:8,000 - 1:500
Saliva	1:32,000 - 1:2,000
Urine	1:8,000 - 1:500
PBMC Cell Culture Supernatant	1:32,000 - 1:2,000
HL-60 Cell Extract	31.25 - 500 ng/mL
Colon Tissue Extract	12.5 - 200 ng/mL

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:4,000 into 1X Wash Buffer PT and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:4,000 for plasma (citrate) or 1:1,000 for plasma (EDTA) into 1X Wash Buffer PT and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (heparin) samples.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect Dilute samples at least 1:2,000 into 1X Wash Buffer PT and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris Dilute samples at least 1:500 into 1X Wash Buffer PT and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:2,000 into 1X Wash Buffer PT and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute samples at least 1:500 into 1X Wash Buffer PT and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Wash Buffer PT.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Wash Buffer PT.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Wash Buffer PT.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

   Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human Calprotectin.

The standard protein in this kit is recombinant full-length Calprotectin (S100A8:S100A9 heterodimer).

Native signal was detected in serum, plasma (citrate), plasma (EDTA), cell culture supernatant, urine, saliva, milk, cell extract, and tissue extract sample types.

CSF samples have not been tested with this kit.

This kit is incompatible with plasma (heparin) samples.

CROSS REACTIVITY
50 ng/mL of recombinant Human S100A8 and 50 ng/mL of recombinant Human S100A9 were individually tested for cross reactivity. No cross reactivity was observed with S100A8 and 0.2% cross reactivity was observed with S100A9.

INTERFERENCE
Recombinant human RAGE, TLR4, S100A8, and S100A9 were individually tested for interference with 500 pg/mL recombinant human Calprotectin. The % decrease in human Calprotectin signal is shown below.

Tested Interferent	Concentration (pg/mL)	% Decrease in Calprotectin Signal
Human RAGE	50,000	4%
Human TLR4	50,000	0%
Human S100A8	50,000	8%
Human S100A8	2,000	4%
Human S100A9	50,000	7%
Human S100A9	2,000	0%

SPECIES REACTIVITY
Other species reactivity was determined by measuring 1:2,000 serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

No signal was observed for the following species: Monkey, Mouse, Rat, Cow, Rabbit, and Goat.

Other species reactivity not determined.

CALCULATION
- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g., linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D
	1	2	
0	0.051	0.053	0.052
31.25	0.090	0.094	0.092
62.5	0.147	0.138	0.143
125	0.219	0.235	0.227
250	0.392	0.409	0.401
500	0.767	0.793	0.780
1,000	1.473	1.423	1.448
2,000	2.834	2.887	2.861

Table 1. Example of human Calprotectin standard curve in 1X Wash Buffer PT. The Calprotectin standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES
Sensitivity:

The calculated minimal detectable dose (MDD) is 13.62 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of Calprotectin were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1:20,000 Serum	107	98 - 112
1:20,000 Plasma – Citrate	105	101 - 109
1:5,000 Plasma – EDTA	100	99 - 101
1:5,000 Stimulated PBMC supernatant	94	85 - 101
1:2,500 Urine	105	105 - 106
1:10,000 Saliva	95	85 - 105
1:2,000 Milk	113	104 - 119
200 ng/ml HL-60 Cell Extract	105	96 - 110
50 ng/mL Human Colon Tissue Extract	106	99 - 113

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Calprotectin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Wash Buffer PT.

Dilution Factor	Interpolated value	1:4,000 Human Serum	1:4,000 Human Plasma (Citrate)	1:1,000 Human Plasma (EDTA)
Undiluted	pg/mL	1,859.1	1,365.7	960.1
	% Expected value	100	100	100
2	pg/mL	896.2	719.7	476.9
	% Expected value	96	105	99
4	pg/mL	462.1	415.2	259.1
	% Expected value	99	122	108
8	pg/mL	243.3	179.1	123.1
	% Expected value	105	105	103
16	pg/mL	122.8	91.2	55.2
	% Expected value	106	107	92

Native Calprotectin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Wash Buffer PT.

Dilution Factor	Interpolated value	1:500 Human Milk	1:2,000 Human Saliva	1:500 Human Urine	1:2,000 PBMC CM
Undiluted	pg/mL	898.7	1,628.8	1,308.5	819.2
	% Expected value	100	100	100	100
2	pg/mL	437.3	784.8	631.1	403.7
	% Expected value	97	96	96	99
4	pg/mL	235.3	381.4	328.8	198.0
	% Expected value	105	94	101	97
8	pg/mL	118.6	209.8	163.2	97.1
	% Expected value	106	103	100	95
16	pg/mL	53.7	91.5	76.6	49.3
	% Expected value	898.7	90	94	96

Dilution Factor	Interpolated value	500 ng/ml HL-60 Cell Extract	200 ng/ml Colon Tissue Extract
Undiluted	pg/mL	989.3	1,119.5
	% Expected value	100	100
2	pg/mL	505.6	533.2
	% Expected value	102	95
4	pg/mL	245.2	283.2
	% Expected value	99	101
8	pg/mL	126.7	139.1
	% Expected value	102	99
16	pg/mL	57.8	69.6
	% Expected value	94	100

Precision

Mean coefficient of variations of interpolated values of Calprotectin from a single concentration of human plasma (EDTA) within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.5	4.4

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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