

ab309184 – Human ICAM1 CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of ICAM1 in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and urine.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab309184

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.

Limitations: All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab309184).

Materials Supplied

Item	Quantity	Storage Condition
Human ICAM1 Capture Antibody 10X	600 µL	+4°C
Human ICAM1 Detector Antibody 10X	600 µL	+4°C
Human ICAM1 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H ₂ O ₂ , 3%)	50 µL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

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

Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.

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).

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 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 4BI. 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 4BI. Mix thoroughly and gently.

CatchPoint HRP Development Solution: Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer. For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. 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 ICAM1 standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 35.2 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 12.
3. Add 150 µL of Sample Diluent NS into tubes number 1-12.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #12 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	150	150	35200	17600
2	Standard#1	150	150	17600	8800
3	Standard#2	150	150	8800	4400
4	Standard#3	150	150	4400	2200
5	Standard#4	150	150	2200	1100
6	Standard#5	150	150	1100	550
7	Standard#6	150	150	550	275
8	Standard#7	150	150	275	137.5
9	Standard#8	150	150	137.5	68.75
10	Standard#9	150	150	68.75	34.38
11	Standard#10	150	150	34.38	17.19
12	Blank Control	0	150	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	1:3200 – 1:200
Plasma - Heparin	1:3200 – 1:200
Plasma - EDTA	1:3200 – 1:200
Plasma - Citrate	1:3200 – 1:200
PBMC Cell Culture Supernatant, Unstimulated	6.25 - 50%
PBMC Cell Culture Supernatant, PHA Stimulated	0.94 - 30%
Urine	12.5 - 50%

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 1:200 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:200 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples at least 1:2 into Sample Diluent NS 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 1:2 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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, resealed 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 prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.
8. Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org.
9. Analyze the data as described below.

Mode	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

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 ICAM1.

The standard protein in this kit is the extracellular domain of human ICAM1.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant and urine.

Spiked protein experiments were used to validate the urine sample type.

Milk, CSF, cell extract, tissue extract samples have not been tested with this kit.

For the measurement of ICAM1 in cell extract sample type, use Human ICAM1 ELISA kit ab309184

SPECIES REACTIVITY

2200 pg/mL of recombinant Mouse ICAM1 was tested for reactivity. No reactivity was observed.

Other species reactivity was determined by measuring 1:200 diluted 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.

Reactivity < 3% was determined for the following species: Monkey, Mouse, Rat, Cow

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices
- Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.
- Create a standard curve by plotting the average blank control subtracted fluorescence 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.
- Note: Most fluorescence 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 fluorescence 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 fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence 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)	RFU		Mean RFU
	1	2	
0	35	47	41
17	103	86	95
34	120	128	124
69	209	237	223
138	464	485	474
275	994	949	971
550	2187	2148	2167
1100	3803	3752	3778
2200	7142	7366	7254
4400	13658	13311	13484
8800	20271	19919	20095
17600	27503	27096	27300

Table 1. Example of human ICAM1 standard curve in Sample Diluent NS. The ICAM1 standard curve was prepared as described in the Standard Preparation section. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 9.9 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of ICAM1 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 (%)
1:400 Serum	91	81 - 100
1:400 Plasma - Heparin	94	87 - 101
1:400 Plasma - Citrate	83	76 - 92
1:400 Plasma - EDTA	89	85 - 95
50% Urine	95	89- 101
50% PBMC Cell Culture Supernatant, Unstimulated	90	89 - 91

*Media is RPMI 1640 containing 10% fetal calf serum.

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 ICAM1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS

Dilution Factor	Interpolated value	1:200 Human Serum	1:200 Human Plasma (Citrate)	1:200 Human Plasma (EDTA)	1:200 Human Plasma (Heparin)	50% PBMC SN, Unstim.	30% PBMC SN, Stim.
Undiluted	pg/mL	1202	1128	1010	940	343	1344
	% Expected value	100	100	100	100	100	100
2	pg/mL	627.0	605	480	462	190	674
	% Expected value	104	107	95	98	111	100
4	pg/mL	338.1	333.3	275.5	275.3	85.1	329.8
	% Expected value	112	118	109	117	99	98
8	pg/mL	163.7	159.3	130.3	130.3	52.1	196.8
	% Expected value	109	113	103	111	122	117
16	pg/mL	74.3	82.7	59.6	58.6	ND	86.9
	% Expected value	99	117	94	100	ND	103

ND – Not Detected – below product dynamic range

Recombinant ICAM1 was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Urine
Undiluted	pg/mL	1558
	% Expected value	100
2	pg/mL	839
	% Expected value	108
4	pg/mL	428.0
	% Expected value	110
8	pg/mL	210.8
	% Expected value	108
16	pg/mL	112.0
	% Expected value	115

Precision

Mean coefficient of variations of interpolated values of ICAM1 from two concentrations of normal human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	6.5	3.1

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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