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ab173194 – IP-10 (CXCL10) Human SimpleStep ELISA™ Kit

For the quantitative measurement of IP-10 (CXCL10) in human serum and plasma samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Abcam's IP-10 (CXCL10) in vitro SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of IP-10 protein in human serum and plasma samples.

The SimpleStep ELISA™ employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

C-X-C motif chemokine 10 (CXCL10 or IP-10) is a small 10.8kD protein that is secreted by several cell types in response to interferon-gamma (IFNg). These cell types include monocytes, endothelial cells and fibroblasts. Upon secretion, CXCL10 is cleaved into an 8.7kD biologically active protein to function in chemotaxis for T-cells, NK cells, monocytes/macrophages and dendritic cells. In addition, CXCL10 has antitumor activity through the inhibition of bone marrow colony formation and angiogenesis. CXCL10 elicits its effects by binding to the cell surface chemokine receptor 3 (CXCR3).

2. Protocol Summary

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed

Add standard or sample to appropriate wells.

Incubate at room temperature.

Add Antibody Cocktail to all wells. Incubate at room temperature.

Aspirate and wash each well. Add TMB Substrate to each well and incubate

Add Stop Solution at a defined endpoint.

Alternatively, record color development kinetically after TMB substrate addition.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Amount	Storage Condition (Before Preparation)
10X IP-10 Capture Antibody	600 µL	+2-8°C
10X IP-10 Detector Antibody	600 µL	+2-8°C
IP-10 Human Lyophilized Recombinant Protein	2 x Vials	+2-8°C
Antibody Diluent CPI	6 mL	+2-8°C
10X Wash Buffer PT	20 mL	+2-8°C
TMB Substrate	12 mL	+2-8°C
Stop Solution	12 mL	+2-8°C
Sample Diluent NS	50 mL	+2-8°C
Pre-coated 96-Well Microplate (12 x 8 well strips)	96 Wells	+2-8°C
Plate Seal	1	+2-8°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
- Cell Extraction Buffer

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

9. 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.

9.1 1X Wash Buffer PT

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

9.2 Antibody cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. 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 CPI. Mix thoroughly and gently.

10. 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).

10.1 Reconstitute the IP-10 protein standard sample by adding 250 µL of sample diluent NS. Mix thoroughly and gently. Hold at room temperature for 3 minutes and mix gently. This is the 5,000 pg/mL **Stock Standard** Solution

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 400 µL of Sample Diluent NS into tube number 1 and 200 µL of Sample Diluent NS into numbers 2-8.

10.4 To prepare tube **#1** add 100 µL from the stock standard into tube **#1.** To prepare tube **#2** add 100 µL from tube **#1** into tube **#2.** Repeat for tubes **#3-7**

10.5 Use the Stock Standard to prepare the following dilution series. Standard **#8** contains no protein and is the Blank control:

Standard #	Volume to dilute (µL)	Volume Diluent NS (µL)	IP-10 (pg/mL)
1	Step 10.4		1000
2	100 µL Standard #1	200	333
3	100 µL Standard #2	200	111
4	100 µL Standard #3	200	37
5	100 µL Standard #4	200	12.4
6	100 µL Standard #5	200	4.1
7	100 µL Standard #6	200	1.4

8 (Blank)	N/A	200	0

11. Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
PBMC conditioned media (+ PHA)	1:7,300 – 1:10
PBMC conditioned media (- PHA)	1:300 - Neat
Human serum	1:50 – Neat
Human plasma (Sodium Citrate)	1:50 – Neat
Human plasma (EDTA)	1:50 – Neat
Human plasma (Heparin)	1:50 – Neat

11.1 Plasma:

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

11.2 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 into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

Refer to Dilution Guidelines for further instruction.

12. 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.

13. Assay Procedure

- Prepare all reagents, working standards, and samples as directed in the previous sections.
- 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.

13.1 Add 50 µL of all sample or standard to appropriate wells.

13.2 Add 50 µL of the Antibody Cocktail to each well.

13.3 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.4 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. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.5 Add 100 µL of TMB Substrate to each well and incubate for 15 minutes in the dark on a plate shaker set to 400 rpm.

13.6 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.

Alternative to 13.7 – 13.8: 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 15 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Δ 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.*

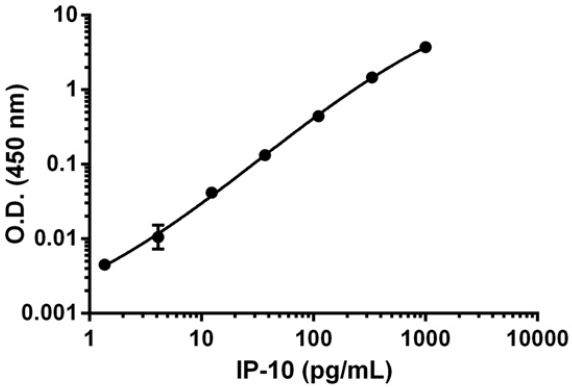
13.9 Analyze the data as described below.

14. Calculations

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. 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			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.103	0.112	0.107
1.4	0.105	0.126	0.115
4.1	0.123	0.132	0.127
12.4	0.151	0.166	0.158
37	0.259	0.256	0.257
111	0.582	0.574	0.578
333	1.696	1.590	1.643
1,000	3.885	3.786	3.835

Figure 1. Example of IP-10 beta standard curve prepared in Sample Diluent NS. The IL-1 beta standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable (MDD) dose is 1.4 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=25) and adding 2 standard deviations then interpolating the corresponding concentrations.

RECOVERY –

Sample Type	Average % Recovery	Range (%)
50% Cell Culture Media	95 - 98	96
10% Human Serum	102 - 105	103
10% Human Plasma (sodium citrate)	93 - 98	96
10% Human Plasma (EDTA)	99 - 103	101
10% Human Plasma (heparin)	82 - 88	86
50% Cell Culture Media	95 - 98	96

Linearity of Dilution

Dilution Factor	Interpolated value	Sample Diluent NS	Conditioned Media	Human Serum
Undiluted	pg/mL	571.1	559.1	504.5
	% Expected Value	100	100	100
1:2	pg/mL	317.6	311.6	333.9
	% Expected Value	111	111	132
1:4	pg/mL	149.7	153.7	157.1
	% Expected Value	105	110%	125
1:8	pg/mL	72.3	76.0	76.4
	% Expected Value	101	109	121
1:16	pg/mL	35.1	40.9	38.0
	% Expected Value	98	117	120

NL- Non-Linear

Dilution Factor	Interpolated value	Human Plasma (Citrate)	Human Plasma (EDTA)	Human Plasma (Heparin)
Undiluted	pg/mL	555.5	603.7	492.1
	%	100	100	100
1:2	pg/mL	325.9	309.2	268.1
	%	117	102	109
1:4	pg/mL	154.3	156.9	138.2
	%	111	104	112
1:8	pg/mL	75.9	77.5	68.6
	%	109	103	111
1:16	pg/mL	37.2	36.0	33.8
	%	107	95	110

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of PBMC conditioned media (+/- PHA) within the working range of the assay.

	Intra-assay Precision	Inter-Assay Precision
N=	9	3
CV (%)	5.1	11.1

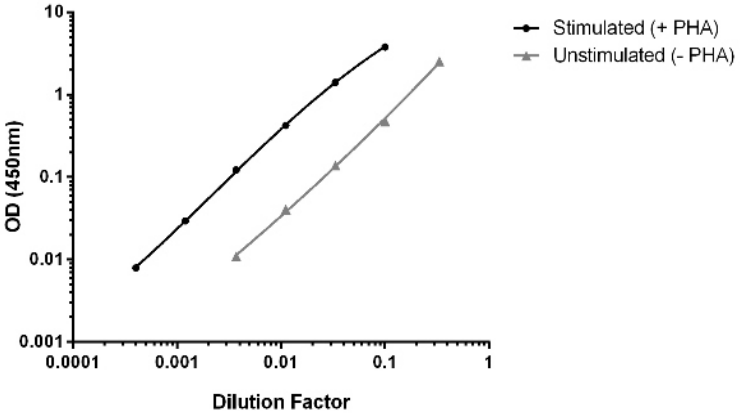


Figure 2. Titration of PBMC conditioned media (+/- PHA) within the working range of the assay. Background subtracted data from triplicate measurements are plotted).

PBMC – Day 2	Range (pg/mL)	Mean (pg/mL)
Unstimulated (-PHA)	1023 - 1102	996
Stimulated (+PHA)	8582 – 10675	9096

Figure 3. Human peripheral blood cells (1x10^6 cells/mL) were cultured in RPMI media supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Conditioned media was harvested after 48 hours, aliquoted and assayed for endogenous IP-10 levels.

17. Assay Specificity

Human Serum	Range (pg/mL)	Mean (pg/mL)
Donor 81	40 - 92	65
Donor 82	47 - 99	74
Donor 83	42 - 93	66
Donor 84	214 - 304	261
Donor 85	53 - 104	78
Donor 86	52 - 107	77
Donor 87	60 - 120	88
Donor 88	43 - 109	75
Donor 89	107 – 173	137
Donor 90	69 - 136	99
Average	65 - 261	102

Figure 4. Observed IP-10 concentration in individual donor normal Human serum samples (n=10). Mean values fall within expected normal reference ranges (Gotsch et al, 2007).

18. Species Reactivity

This kit detects IP-10 in human serum and plasma samples.

Urine and saliva samples have not been tested with this kit.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.

20. Notes

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