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# ab229410
# Human IP-10 (CXCL10) CatchPoint® 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

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

The CatchPoint 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. CatchPoint HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.

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

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes

Read fluorescence at Ex/Cutoff/Em 530/570/590 nm

## 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 handle 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.
- All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab173194).
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## 6. Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Human IP-10 (CXCL10) Capture Antibody 10X | 600 µL | +4°C |
| Human IP-10 (CXCL10) Detector Antibody 10X | 600 µL | +4°C |
| Human IP-10 (CXCL10) Lyophilized Recombinant Protein | 2 Vials | +4°C |
| Antibody Diluent CPI | 6 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 ($H_2O_2$, 3%) | 50 µL | +4°C |
| Sample Diluent NS* | 50 mL | +4°C |
| SimpleStep Pre-Coated Black 96-Well Microplate | 96 Wells | +4°C |
| Plate Seal | 1 | +4°C |

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

## 7. Materials Required, Not Supplied

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

- Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 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).

## 8. Technical Hints

- 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.
- 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.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The incubation times provided in this protocol were optimized for fastest results with good signal. It is possible to increase the signal with longer incubation times, further optimization might be necessary.
- Keep in mind any RFU values shown are relative, NOT absolute. RFU from one plate reader are not comparable to another, especially if different make or model.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- 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.

# 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 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.

## 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.

## 9.3 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.

## 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 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the IP-10 (CXCL10) by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IP-10 (CXCL10) standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 2,500 pg/mL **Stock Standard** Solution.

10.2 Label 12 tubes, Standards 1– 12.

10.3 Add 108 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-12.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #12 contains no protein and is the Blank control.

Standards will be added to the plate in step 13.3. If desired all 9 standards can be used for a full standard curve. Alternatively, to commit fewer wells to the standard curve, select a subset of at least 7 standards plus the blank control. If 7 standards are desired, we recommend standards #2-8.

| Standard # | Dilution Sample | Volume to Dilute (µL) | Volume of Diluent (µL) | Starting Conc. (pg/mL) | Final Conc. (pg/mL) |
|---|---|---|---|---|---|
| 1 | Stock | 192 | 108 | 2,500 | 1,600 |
| 2 | Standard#1 | 150 | 150 | 1,600 | 800 |
| 3 | Standard#2 | 150 | 150 | 800 | 400 |
| 4 | Standard#3 | 150 | 150 | 400 | 200 |
| 5 | Standard#4 | 150 | 150 | 200 | 100 |
| 6 | Standard#5 | 150 | 150 | 100 | 50 |
| 7 | Standard#6 | 150 | 150 | 50 | 25 |
| 8 | Standard#7 | 150 | 150 | 25 | 12.5 |
| 9 | Standard#8 | 150 | 150 | 12.5 | 6.3 |
| 10 | Standard#9 | 150 | 150 | 6.3 | 3.1 |
| 11 | Standard#10 | 150 | 150 | 3.1 | 1.6 |
| 12 | None | 0 | 150 | 0 | 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 -20°C 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 -20°C 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. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

## 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 fluorescence or "edge effects" have not been observed with this assay.
- Ensure plate and all materials are equilibrated to room temperature during use.
- Cover the plate with a plate seal during incubation steps.

## 13. 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.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

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

**13.2** 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.

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

**13.4** Add 50 µL of the Antibody Cocktail to each well.

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

**13.6** 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.

**13.7** 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.

**13.8** 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

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

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

13.9 Analyze the data as described below.

# 14. Calculations

14.1 Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices

14.2 Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.

14.3 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.

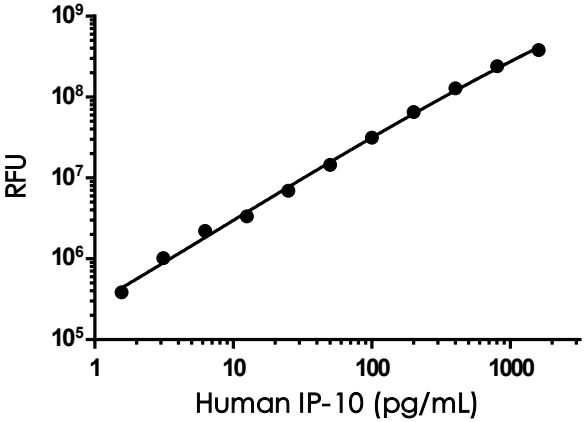
Δ Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.4 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.

14.5 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.

# 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 | | | |
|---|---|---|---|
| Concentration (pg/mL) | RFU | | Mean RFU |
| | 1 | 2 | |
| 0 | 857,792 | 859,496 | 858,644 |
| 1.6 | 1,271,768 | 1,215,550 | 1,243,659 |
| 3.1 | 1,918,196 | 1,839,415 | 1,878,806 |
| 6.3 | 3,008,541 | 3,136,342 | 3,072,442 |
| 12.5 | 4,164,933 | 4,269,184 | 4,217,059 |
| 25 | 7,589,510 | 8,042,170 | 7,815,840 |
| 50 | 15,260,389 | 15,538,503 | 15,399,446 |
| 100 | 31,529,056 | 33,295,040 | 32,412,048 |
| 200 | 66,256,744 | 66,535,996 | 66,396,370 |
| 400 | 124,284,112 | 133,494,352 | 128,889,232 |
| 800 | 240,028,064 | 242,030,096 | 241,029,080 |
| 1,600 | 381,966,176 | 380,825,280 | 381,395,728 |

**Figure 1**. Example of human IP-10 (CXCL10) standard curve in Sample Diluent NS. The IP-10 (CXCL10) standard curve was prepared as described in Section 10. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

# 16. Typical Sample Values

## SENSITIVITY –

The calculated minimal detectable dose (MDD) is 1.1 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

## RECOVERY –

(Sample spiking in representative sample matrices)

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

## 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.

Recombinant IP-10 (CXCL10) was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

| 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** |
| 2 | pg/mL | 317.6 | 311.6 | 333.9 |
| | **% Expected value** | **111** | **111** | **132** |
| 4 | pg/mL | 149.7 | 153.7 | 157.1 |
| | **% Expected value** | **105** | **110%** | **125** |
| 8 | pg/mL | 72.3 | 76.0 | 76.4 |
| | **% Expected value** | **101** | **109** | **121** |
| 16 | pg/mL | 35.1 | 40.9 | 38.0 |
| | **% Expected value** | **98** | **117** | **120** |

Recombinant IP-10 (CXCL10) was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

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

**PRECISION** –

Mean coefficient of variations of interpolated values of IP-10 (CXCL10) from three concentrations of PBMC conditioned media (+/- PHA) within the working range of the assay.

| | Intra-Assay | Inter-Assay |
|---|---|---|
| n = | 9 | 3 |
| CV(%) | 5.1 | 11.1 |

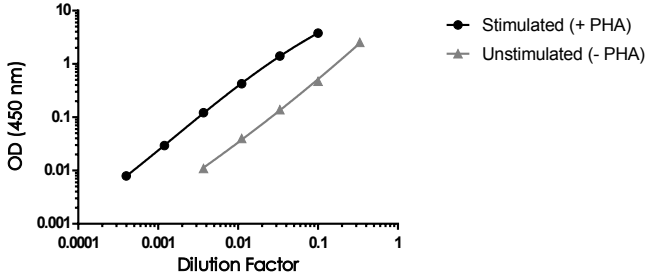



**Figure 2.** Titration of PBMC conditioned media (+/- PHA) within the working range of the assay. Background subtracted data from triplicate measurements are plotted

| 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 recognizes human IP-10 (CXCL10) 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 |
|---|---|---|
| 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 CatchPoint HRP Development Solution too brief | Read plate again after longer incubation time |
| 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 Stoplight Red Substrate protected from light. |
| Precipitate in Diluent | Precipitation and/or coagulation of components within the Diluent. | Precipitate can be removed by gently warming the Diluent to 37°C. |

# 20. Notes

## Technical Support



### For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)