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Human IP-10 / CXCL10 ELISA Set

Catalog No: CDK336A, B, C, D **Quantity:** 5, 10, 15, 20 x 96 tests

Specificity:	Native and recombinant human IP-10
Sensitivity:	< 5.7 pg/ml
Range:	200 to 6.25 pg/ml
Sample Type:	Cell supernatants, buffered solutions, serum, plasma samples and other bodily fluids.
Cross-Reactivity:	No cross reactivity with other human molecules.

1. INTRODUCTION

The Cell Sciences® IP-10 ELISA Set is intended for use in a 'do it yourself' solid phase sandwich ELISA for the *in vitro* qualitative and quantitative determination of IP-10 in supernatants, buffered solutions, serum, plasma samples and other body fluids.

This assay will recognize both native and recombinant human IP-10.

Principle of the ELISA method

A capture antibody highly specific for IP-10 is coated to the wells a microtiter strip plate. Binding of IP-10 in samples and known standards to the capture antibodies is completed and then any excess unbound analyte is removed.
During the next incubation period the binding of the biotinylated anti- IP-10 secondary antibody to the analyte occurs. Any excess unbound secondary antibody is then removed.
The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of IP-10 present in the samples and standards.
The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IP-10 in any sample tested.

2. BACKGROUND

IP-10 (Interferon-gamma inducible Protein 10kDa) also known as CXCL10, is secreted by several cell types in response to IFNγ and LPS. These cell types include monocytes, endothelial cells and fibroblasts. The gene for IP-10 is located on chromosome 4 in a cluster among several other cytokines and encodes a 98 amino acid precursor protein.
Several roles have been attributed to IP-10, such as chemoattraction for monocytes and T cells (but not for neutrophils), inhibition of bone marrow colony formation and angiogenesis, promotion of T cells adhesion molecule expression.
IP-10 shares a common receptor, CXCR3, with the chemokine MIG, but has been shown to play a distinct role in host defense in infections.
IP-10 expression has been associated with HIV infection, is involved in inflammatory skin disease and other allergic diseases; it appears in inflammation of the nervous system and in Alzheimer's disease (astrocytes expressing IP-10 are commonly associated with senile plaques.

Cell Sciences
65 Parker Street, Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

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Web Site: www.cellsciences.com

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3. REAGENTS PROVIDED: RECONSTITUTION & STORAGE GUIDELINES

REAGENTS (Store at 2-8°C)	5 x 96 wells	10 x 96 wells	15 x 96 wells	20 x 96 wells	RECONSTITUTION * STORAGE (see Section 9 – Assay Preparation)
A. IP-10 Standard: 200 pg/ml (lyophilized)	5 vials	10 vials	15 vials	20 vials	**Reconstitute as directed on the vial.** *Discard reconstituted standard after use.*
B. Capture Antibody (0.25 ml/ vial)	1 vial	2 vials	3 vials	4 vials	Sterile. Dilute prior to use. *Discard diluted antibody after use.*
C. Biotinylated anti-IP-10 Detection Antibody (lyophilized)	1 vial	2 vials	3 vials	4 vials	Reconstitute with **0.55 ml** Reconstitution Buffer prior to use. *Store reconstituted detection antibody at 2-8°C for 1 year.*
D. Streptavidin-HRP (25 µl/ vial)	1 vial	2 vials	3 vials	4 vials	Dilute prior to use. *Discard diluted Streptavidin-HRP conjugate after use.*
E. TMB Substrate (25 ml/ vial)	2 vials	4 vials	6 vials	8 vials	Ready-to-use.

4. MATERIALS REQUIRED BUT NOT PROVIDED. *Available in 5-plate Accessory Pack for ELISA, CDK338

- 96-well Microtiter plates (recommend Nunc Maxisorp)
- Reconstitution Buffer (1x PBS + 0.09% Azide). *Store at 2-8°C for up to 1 week.**
- Coating Buffer (1x PBS, pH 7.2-7.4). *Store at 2-8°C for up to 1 week*.*
- Wash Buffer (1x PBS + 0.05% Tween20). *Use immediately.**
- Blocking Buffer (1x PBS + 5% BSA). *Store at 2-8°C for up to 1 week*.*
- Standard and Detection Antibody Dilution Buffer (1x PBS + 1% BSA). *Store at 2-8°C for up to 1 week.**
- HRP Diluent Buffer (1x PBS + 1% BSA + 0.1% Tween20). *Store at 2-8°C for up to 1 week.**
- Stop Reagent (1M Sulfuric Acid)
- Microtiter plate reader with appropriate filters (450nm required with optional 620nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000 µl adjustable single channel micropipettes with disposable tips
- 50-300 ml multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile.

5. STORAGE INSTRUCTIONS

Store the kit reagents between 2 and 8°C. Immediately after use, remaining reagents should be returned to cold storage (2-8°C). Expiration date of the kit and reagents is stated on box front labels. The expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated during handling.

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6. SAFETY AND PRECAUTIONS FOR USE

- FOR RESEARCH ONLY. Not for diagnostic use.
- Handling of reagents, serum, or plasma specimens should be in accordance with local safety procedures (e.g., CDC/NIH Health manual: “Biosafety in Microbiological and Biomedical Laboratories" 1984.)
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated or frozen, as indicated.
- All reagents should be warmed to room temperature before use.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean, disposable, plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination.
- For the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual wash liquid must be drained from the wells by efficient aspiration, or by decantation, followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- **Warning**: TMB is toxic avoid direct contact with hands. Dispose of properly.
- TMB solution is light sensitive. Avoid prolonged exposure to light.
- Avoid contact of TMB solution with metal to prevent color development. If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.
- Read absorbances within 1 hour after completion of the assay.

7. SPECIMEN COLLECTION, PROCESSING & STORAGE

Cell culture supernatants, human serum, plasma or other biological samples are suitable for use in the assay. If not analyzed shortly after collection, samples should be aliquoted (250-500 µl) to avoid repeated freeze-thaw cycles and stored frozen at –80°C. Avoid multiple freeze-thaw cycles of frozen specimens. DO NOT thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use.

Cell supernatants: Remove particulates and aggregates by centrifugation at 1000 x g for 10 minutes.

Serum: Remove serum from the clot or red cells as soon as possible after clotting and separation, respectively. When possible avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, these should be removed prior to use

Plasma: EDTA, citrate and heparin plasma can be assayed. Centrifuge samples at 1000 x g for 30 min to remove particulates. Harvest plasma.

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8. PLATE PREPARATION

For 1 96-well plate, add 100 µl of Capture Antibody to 10 ml of Coating Buffer. *Discard diluted antibody after use.*

1.	Addition	Add 100 µl of diluted **Capture Antibody** to each well.
2.	Incubation	Cover with a plastic plate cover and incubate at **4°C overnight**.
3.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.4 ml of **Wash Solution** into each well. c) Aspirate the contents of each well. d) Repeat steps b and c.
4.	Addition	Add 250 µl of **Blocking Buffer** to each well.
5.	Incubation	Cover with a plastic plate cover and incubate at **room temperature (18 to 25°C) for 2 hours.**
6.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.4 ml of Wash Solution into each well. c) Aspirate the contents of each well. d) Repeat steps b and c twice more.
For immediate use of the plate, continue to Section 9. To store the coated and blocked plates for future use, dry the plate on the bench at room temperature for 24 hours. Cover the plates and store at 2-8°C in a sealed plastic bag with desiccant for up to 12 months.		

9. ASSAY PREPARATION

- **Bring all reagents to room temperature before use.**

Assay Design

Determine the number of microwell strips required to test the desired number of samples, plus the number of wells needed for blanks and standards. Each sample, standard and blank should be tested **in duplicate.**

Example Plate Layout:

	Standards (pg/ml)		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	200	200										
B	100	100										
C	50	50										
D	25	25										
E	12.5	12.5										
F	6.25	6.25										
G	blank	blank										
H												

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Preparation of Standard

Standard vials must be reconstituted with the **volume of Standard Dilution Buffer shown on the vial immediately prior to use.** This reconstitution gives a stock solution of 200 pg/ml of IP-10. Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 200 to 6.25 pg/ml. *Discard reconstituted standard after use*

Note: A fresh standard curve should be produced for each new assay.

1. Immediately after reconstitution add 200 µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 200 pg/ml.
2. Add 100 µl of appropriate Standard Dilution Buffer to the remaining standard wells B1/ B2 through F1/ F2.
3. Transfer 100 µl from wells A1 to B1 and A2 to B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
4. Continue this 1:1 dilution using 100 µl from wells B1 and B2 through to wells F1 and F2 providing a serially diluted standard curve ranging from 200 pg/ml to 6.25 pg/ml.
5. Discard 100 µl from the final wells of the standard curve (F1 and F2).

Alternatively, these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

Preparation of Biotinylated anti-IP-10 Detection Antibody

It is recommended this reagent is prepared **immediately before use**. Reconstitute with 0.55 ml Reconstitution Buffer prior to use. *Store reconstituted detection antibody at 2-8°C for 1 year.* Dilute the reconstituted biotinylated anti-IP-10 with the Biotinylated Antibody Dilution buffer in an appropriate clean glass vial. For one plate, add 100 µl of the reconstituted Detection Antibody into 5 ml of Biotinylated Antibody dilution buffer.

Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a micro-centrifuge to collect the volume at the bottom.

Dilute 5 µl of Streptavidin-HRP into 0.5 ml of HRP Diluent Buffer **immediately before use**.

To prepare the working solution of Streptavidin-HRP conjugate, further dilute 150 µl of the diluted HRP solution into 10 ml of HRP Diluent Buffer.

Discard diluted Streptavidin-HRP conjugate after use.

10. ASSAY METHOD

- **We strongly recommend that every vial is mixed thoroughly without foaming prior to use, except the standard vial which must be mixed gently by inversion only.**

Note: Prepare Biotinylated Detection Antibody and Streptavidin-HRP immediately before use.

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Assay Step	Details
1. Preparation	Prepare **Standard curve** as shown in Section 9.
2. Addition	Add 100 µl of each **Standard, sample, blank** (Standard Dilution Buffer) to appropriate wells in duplicate.
3. Incubation	Cover with a plastic plate cover and incubate at **room temperature (18-25°C) for 2 hours**.
4. Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.4 ml of **Wash Solution** into each well. c) Aspirate the contents of each well. d) Repeat step b and c.
5. Addition	Add 50 µl of diluted **Detection Antibody** into all wells.
6. Incubation	Cover with a plastic plate cover and incubate at **room temperature (18-25°C) for 1 hour**.
7. Wash	Repeat wash step 4.
8. Addition	Add 100 µl of Streptavidin-**HRP** solution into all wells.
9. Incubation	Cover with a plastic plate cover and incubate at **room temperature (18-25°C) for 30 min**.
10. Wash	Repeat wash step 4.
11. Addition	Add 100 µl of ready-to-use **TMB Substrate Solution** into all wells.
12. Incubation	Incubate in the **dark for 5-15 minutes* at room temperature**. Avoid direct exposure to light by wrapping the plate in aluminum foil.
13. Addition	Add 100 µl of **H_2SO_4 Stop Reagent** into all wells.
Read the absorbance value of each well immediately after step 13 on a spectrophotometer using 450 nm as the primary wavelength and 620 nm as the reference wavelength (610 nm to 650 nm are acceptable).	

**Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore, the color development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*

11. DATA ANALYSIS

Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean. Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding standard concentration on the horizontal axis. The amount of IP-10 in each sample is determined by extrapolating OD values against IP-10 standard concentrations using the standard curve.

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12. ASSAY LIMITATIONS

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard Diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples, always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

13. PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity, minimum detectable dose of this IP-10 ELISA Set was determined using the IP-10 ELISA kit (which contains the same antibodies) and was found to be **< 5.7 pg/ml**. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 32 times.

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