Quantikine® ELISA

Human CXCL10/IP-10 Immunoassay

Catalog Number DIP100
SIP100
PDIP100

For the quantitative determination of human Interferon gamma inducible Protein 10 (IP-10) concentrations in cell culture supernates, serum, plasma, saliva, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

IP-10 (interferon-gamma inducible protein 10 kDa), also known as CXCL10, was originally identified as an IFN-γ-inducible gene. It is induced in a variety of cells in response to IFN-γ and LPS. In contrast to other CXC chemokines, IP-10 has no chemotactic activity for neutrophils. It is a pleiotropic molecule that appears to target activated T cells and monocytes (1-3). IP-10 inhibits bone marrow colony formation and angiogenesis (4, 5). It can also stimulate NK and T cell migration, regulate T cell maturation and modulate adhesion molecule expression (for a review, see reference 6).

IP-10 cDNA encodes a 98 amino acid (aa) precursor protein with a 21 aa signal peptide that is cleaved to generate a 77 aa mature protein (1). The aa sequence of IP-10 indicates that it is a member of a subfamily of CXC chemokines lacking the ELR domain.

CXCR3 is a receptor for both IP-10 and MIG (7, 8). It is highly expressed in IL-2-activated T lymphocytes and can also be expressed on eosinophils (9), yet is undetectable in resting T lymphocytes, B lymphocytes, monocytes or granulocytes. CXCR3 can also be expressed on $CD34^+$ hematopoietic progenitors from human cord blood stimulated with GM-CSF, but not on freshly isolated $CD34^+$ progenitor cells (10). CXCR3 promotes Ca^{2+} mobilization and chemotaxis specifically in response to IP-10 and MIG, and not to other CXC or CC chemokines (7).

IP-10 mRNA is expressed by activated T lymphocytes, neutrophils, splenocytes, keratinocytes, osteoblasts, astrocytes, endothelial cells, and smooth muscle cells (11). It is also expressed in inflammatory skin diseases and cutaneous T cell lymphomas.

IP-10 expression has been associated with HIV infection. It can contribute to the accumulation of activated T cells in the cerebrospinal fluid compartment in HIV-1 infected individuals (12). The retroviral transactivator, HIV-1 Tat, is a potent inducer of IP-10 expression in astrocytes (13). Tat can induce expression levels of IP-10 sufficient to promote chemotaxis of peripheral blood lymphocytes. This Tat-mediated IP-10 mRNA induction can be suppressed by a mitogen-activated protein kinase (MAPK) inhibitor, thus indicating that MAPKs play a major role in Tat-mediated chemokine induction in astrocytes.

IP-10 expression has also been shown to be significantly elevated in astrocytes within the brains of Alzheimer's Disease patients (14). Astrocytes expressing IP-10 are commonly associated with senile plaques. The receptor for IP-10, CXCR3, can be detected constitutively on neurons and neuronal processes in various cortical and subcortical regions of the brain.

The Quantikine® Human CXCL10/IP-10 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human IP-10 in cell culture supernates, serum, plasma, saliva, and urine. It contains *E. coli*-expressed recombinant human IP-10 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IP-10 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IP-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IP-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IP-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IP-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IP-10 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the appropriate calibrator diluent.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DIP100	CATALOG # SIP100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IP-10 Microplate	890834	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IP-10.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IP-10 Standard	890836	1 vial	6 vials	Recombinant human IP-10 in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C for up to 1 month in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human IP-10 Conjugate	890835	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IP-10 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-56	895102	1 vial	6 vials	17 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate/saliva/urine samples.*	
Calibrator Diluent RD6Q	895128	1 vial	6 vials	21 mL/vial of buffered animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DIP100 contains sufficient materials to run an ELISA on one 96 well plate.
SIP100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDIP100). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the Pharmpak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL, and not in the glass vials described in the package insert. **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human IP-10 Microplate	890834	50 plates
Human IP-10 Standard	890836	25 vials
Human IP-10 Conjugate	890835	50 vials
Assay Diluent RD1-56	895102	50 vials
Calibrator Diluent RD5K	895119	50 vials
or		
Calibrator Diluent RD6Q	895128	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Insert	750518	2 booklets

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards and samples.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent.
- Human IP-10 Controls (optional; R&D Systems®, Catalog # QC23).

PRECAUTIONS

Calibrator Diluent RD6Q contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

IP-10 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running the assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

Saliva - Collect saliva using a collection device such as a Salivette® or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Most cell culture supernate samples require at least a 30-fold dilution. A suggested 30-fold dilution is 10 µL of sample + 290 µL of Calibrator Diluent RD5K.

All saliva samples require at least a 10-fold dilution. A suggested 10-fold dilution is 25 µL of sample + 225 µL of Calibrator Diluent RD5K.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

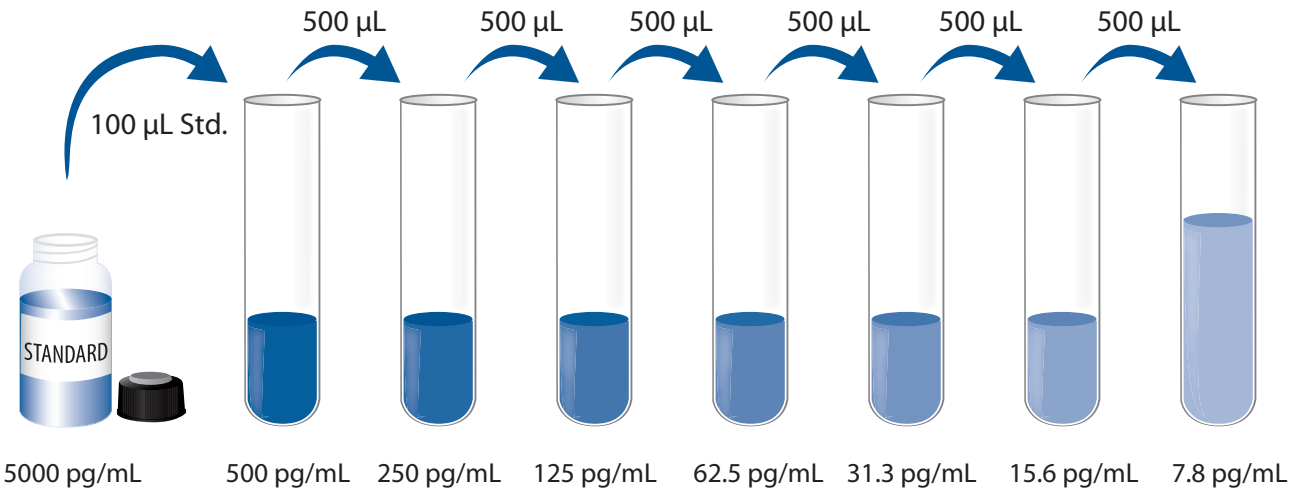
Note: *High concentrations of IP-10 are found in saliva. Take precautionary measures to prevent contamination of kit reagents.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human IP-10 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IP-10 Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5K (*for cell culture supernate/saliva/urine samples*) or Calibrator Diluent RD6Q (*for serum/plasma samples*) into the 500 pg/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: *High concentrations of IP-10 are found in saliva. Take precautionary measures to prevent contamination of kit reagents.*

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, and reseal.
3. **For Cell Culture Supernate/Saliva/Urine samples:** Add 150 µL of Assay Diluent RD1-56 to each well.
   For Serum/Plasma samples: Add 75 µL of Assay Diluent RD1-56 to each well.
4. **For Cell Culture Supernate/Saliva/Urine samples:** Add 100 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
   For Serum/Plasma samples: Add 75 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
6. Add 200 µL of Human IP-10 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

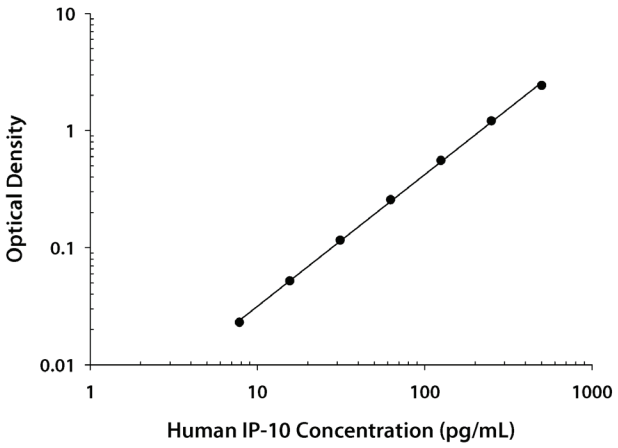
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IP-10 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

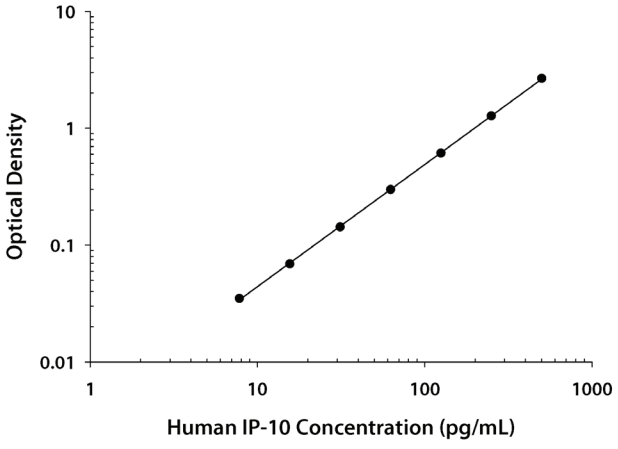
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.027	0.027	—
7.8	0.052 0.048	0.050	0.023
15.6	0.082 0.076	0.079	0.052
31.3	0.145 0.141	0.143	0.116
62.5	0.288 0.281	0.284	0.257
125	0.575 0.588	0.582	0.555
250	1.251 1.215	1.233	1.206
500	2.455 2.460	2.458	2.431

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.027	0.027	—
7.8	0.064 0.061	0.062	0.035
15.6	0.101 0.090	0.096	0.069
31.3	0.174 0.166	0.170	0.143
62.5	0.338 0.314	0.326	0.299
125	0.652 0.629	0.640	0.613
250	1.314 1.288	1.301	1.274
500	2.622 2.764	2.693	2.666

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	70.3	174	342	80.7	194	362
Standard deviation	2.07	5.15	10.74	7.91	13.48	24.27
CV (%)	2.9	3.0	3.1	9.8	6.9	6.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	67.4	168	334	70.7	182	366
Standard deviation	3.12	4.99	11.15	6.23	11.12	18.93
CV (%)	4.6	3.0	3.3	8.8	6.1	5.2

RECOVERY

The recovery of human IP-10 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	95	88-101%
Serum (n=5)	99	88-112%
EDTA plasma (n=5)	99	90-109%
Heparin plasma (n=5)	99	87-113%

SENSITIVITY

Fifty-seven assays were evaluated and the minimum detectable dose (MDD) of human IP-10 ranged from 0.41-4.46 pg/mL. The mean MDD was 1.67 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IP-10 were serially diluted with the calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=9)	Serum (n=5)	EDTA plasma (n=9)	Heparin plasma (n=9)	Saliva (n=5)	Urine (n=4)
1:2	Average % of Expected	102	102	104	106	110	103
	Range (%)	95-106	92-107	95-113	101-112	106-118	101-106
1:4	Average % of Expected	102	106	104	108	112	106
	Range (%)	96-106	93-117	98-111	101-113	108-120	102-110
1:8	Average % of Expected	103	106	98	106	112	109
	Range (%)	97-107	99-112	94-111	97-112	108-127	101-117
1:16	Average % of Expected	102	106	102	105	117	110
	Range (%)	92-112	99-115	83-108	98-112	117-117	98-121

CALIBRATION

This immunoassay is calibrated against a highly purified *E-coli*-expressed recombinant human IP-10 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human IP-10 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=60)	89	38-361
EDTA plasma (n=35)	96	47-382
Heparin plasma (n=35)	110	52-450
Saliva (n=5)	729	292-1340

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=18)	17.2	67	ND-49.7

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 mM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human IP-10.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	29,774	21,900
Stimulated	18,456	11,091

THP-1 human acute monocyte leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated with 1.0 µg/mL recombinant human IFN-γ for 8 hours and then 1.0 µg/mL LPS was added. Cells were incubated for an additional 18 hours. An aliquot of the cell culture supernate was removed, diluted 600-fold and assayed, and measured 164,640 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IP-10.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human IP-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
BLC/BCA-1
ENA-78
GCP-2
GROα
GROβ
GROγ
IFN-γ
IL-8
IL-8, endothelial cell-derived
I-TAC
MIG
NAP-2
SDF-1α
SDF-1β

Recombinant mouse:
BLC/BCA-1
CRG-2 (IP-10)
GCP-2
KC
MIG
SDF-1α

Recombinant porcine:
IL-8

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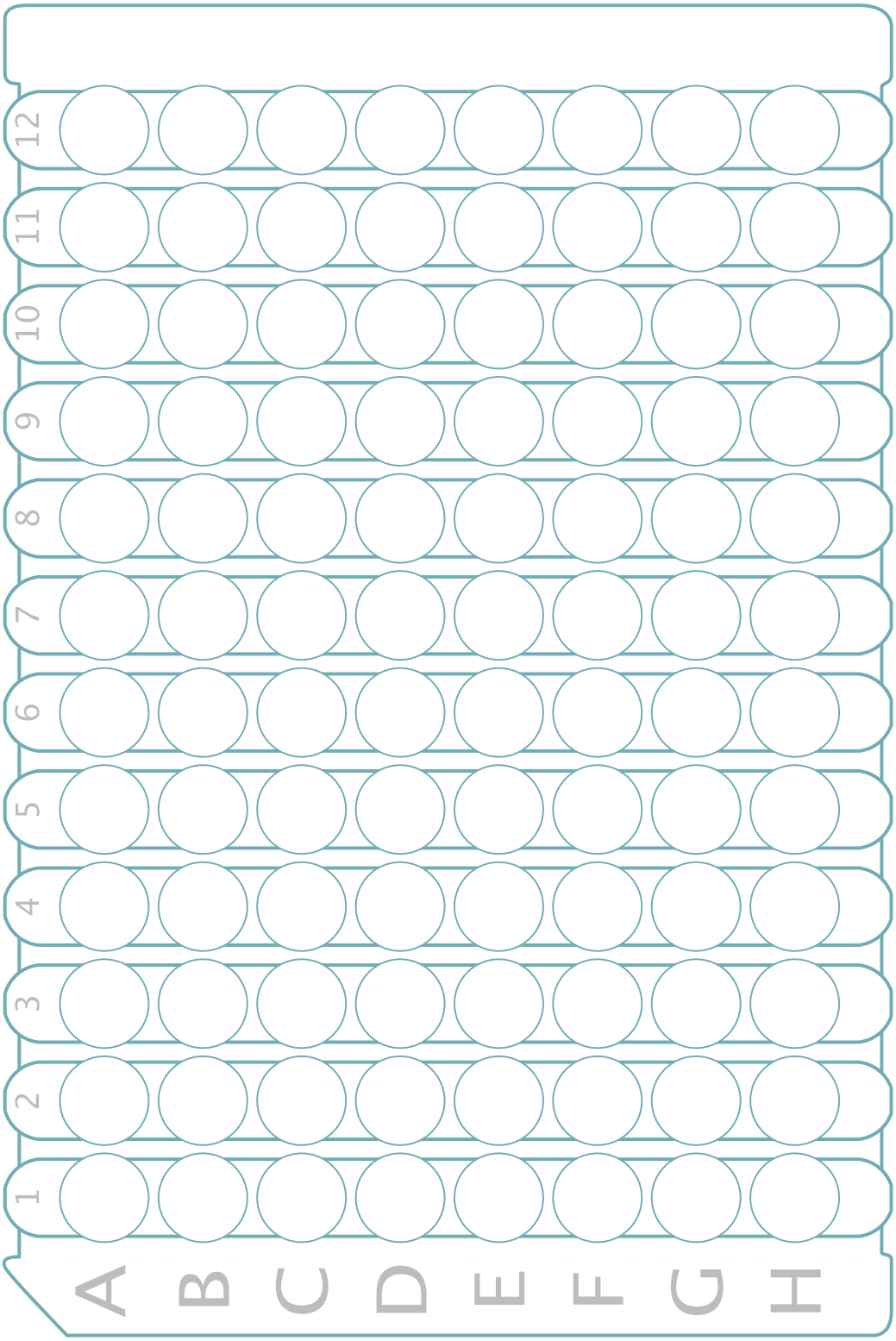
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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