Quantikine® QuicKit™ ELISA

Human CXCL10/IP-10 Immunoassay

Catalog Number QK266

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

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

TABLE OF CONTENTS

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

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® QuicKit™ Human CXCL10/IP-10 Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human IP-10 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IP-10 and antibodies raised against the recombinant protein. Results obtained using natural human IP-10 showed linear curves that were parallel to the standard curves obtained using the QuicKit™ 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. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked polyclonal detection antibody, specific for human IP-10. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of IP-10 bound. 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.
- If samples generate values higher than the highest standard, dilute the samples with the calibrator diluent and repeat the assay.
- 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® QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuicKit™ Coated Microplate	899063	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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	899147	2 vials of recombinant human IP-10 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IP-10 Capture Ab Concentrate	899145	Lyophilized tagged monoclonal antibody specific for human IP-10.	May be stored for up to 1 month at 2-8 °C.*
Human IP-10 Detection Ab Concentrate	899146	400 µL of a polyclonal antibody specific for human IP-10 conjugated to horseradish peroxidase with preservatives.	
Calibrator Diluent RD5-76	896235	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

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
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Human IP-10 Controls (optional; R&D Systems®, Catalog # QC271)

PRECAUTIONS

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.*

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution due to a matrix effect. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-76.

Some supernates may require dilution due to high endogenous levels. Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

Human IP-10 Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IP-10 Capture Ab Concentrate with Calibrator Diluent RD5-76. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

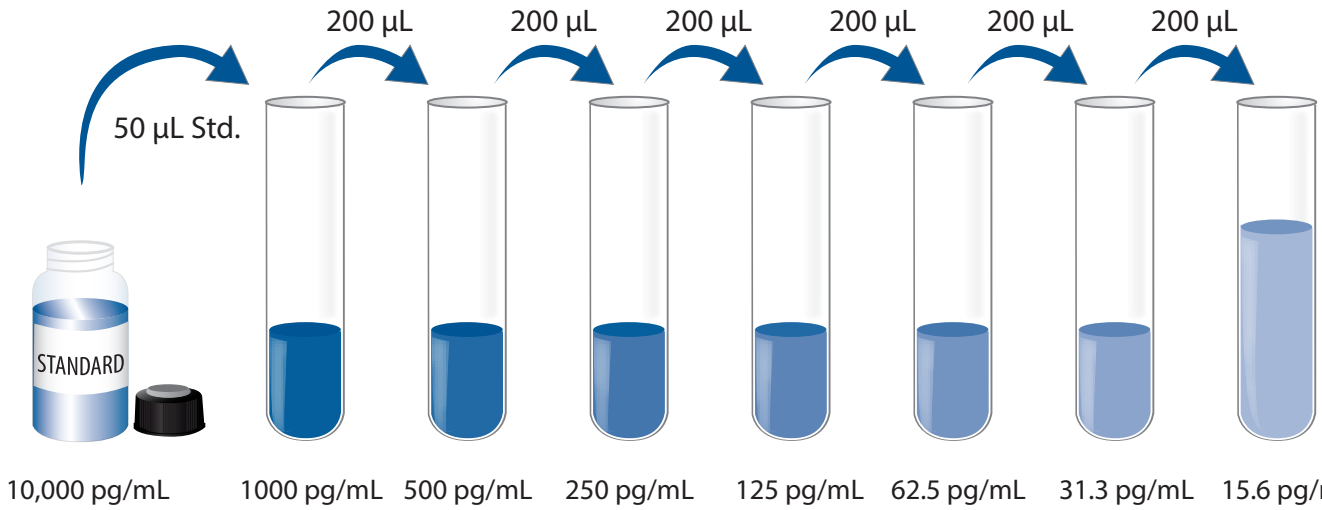
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Calibrator Diluent RD5-76. For a full plate, add 300 µL of reconstituted Human IP-10 Capture Ab stock and 300 µL of Human IP-10 Detection Ab Concentrate to 5.4 mL of Calibrator Diluent RD5-76.

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. 100 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 Calibrator Diluent RD5-76. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-76 into the 1000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-76 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: *IP-10 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, samples, and working standards 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. Add 50 µL of standard, control, or sample per well.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. 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 towels.
6. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. 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.
8. 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.

CALCULATION OF RESULTS

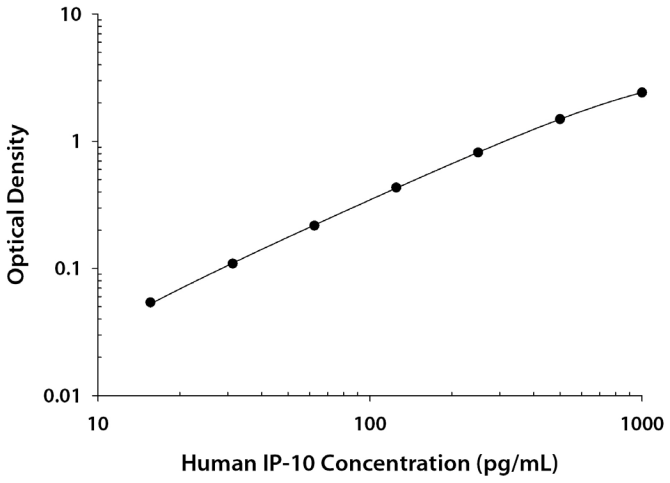
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph. The data may be linearized by plotting the log of the human IP-10 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.008 0.009	0.009	——
15.6	0.061 0.065	0.063	0.054
31.3	0.116 0.119	0.118	0.109
62.5	0.222 0.230	0.226	0.217
125	0.432 0.449	0.441	0.432
250	0.823 0.829	0.826	0.817
500	1.495 1.508	1.502	1.493
1000	2.427 2.428	2.428	2.419

PRECISION

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

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	**1**	**2**	**1**	**2**
n	20	20	10	10
Mean (pg/mL)	96.7	521	95.3	515
Standard deviation	1.12	10.6	10.8	42.1
CV (%)	1.2	2.0	11.3	8.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	119	110-129%
Serum* (n=2)	71	67-76%
EDTA plasma* (n=2)	86	78-95%
Heparin plasma* (n=2)	104	92-118%

*Samples were diluted prior to assay.

SENSITIVITY

Eleven assays were evaluated and the minimum detectable dose (MDD) of human IP-10 ranged from 0.476-4.10 pg/mL. The mean MDD was 1.79 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. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)
1:2	Average % of Expected	95	107	102	94
	Range (%)	92-98	105-109	100-104	91-96
1:4	Average % of Expected	94	118	107	93
	Range (%)	89-99	115-121	103-111	90-97
1:8	Average % of Expected	94	128	110	93
	Range (%)	86-99	124-131	104-116	90-96

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 - 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)	Standard deviation
Serum (n=10)	85.6	49.8-250	62.0
EDTA plasma (n=10)	99.4	60.4-205	51.8
Heparin plasma (n=10)	137	83.9-281	75.0

Cell Culture Supernates:
Human peripheral blood cells (PBMCs) (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were left unstimulated or stimulated with 10 µg/mL PHA for 5 days. Aliquots of the cell culture supernates were removed, assayed for levels of human IP-10, and measured 12,555 pg/mL and 6570 pg/mL, respectively.

PBMCs were cultured in the medium as described above. Cells were left unstimulated or stimulated with 1 µg/mL LPS and 40 ng/mL of recombinant human IFN-γ for 24 hours. Aliquots of the cell culture supernates were removed, assayed for levels of human IP-10, and measured 174 pg/mL and 958 pg/mL, respectively.

THP-1 human acute monocyte leukemia cells were left 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 left unstimulated or stimulated with 1 µg/mL of recombinant human IFN-γ for 8 hours and then 1.0 µg/mL LPS was added. Cells were incubated for an additional 18 hours. Aliquots of the cell culture supernates were removed, assayed for levels of human IP-10, and measured 1172 pg/mL and 270,600 pg/mL, respectively.

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 low level 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

Other recombinants:

feline SDF-1β
porcine IL-8
rat CXCL10/IP-10

REFERENCES

1. Luster, A.D. *et al.* (1985) Nature **315**:672.
2. Taub, D.D. *et al.* (1993) J. Exp. Med. **177**:1809.
3. Taub, D.D. *et al.* (1995) J. Immunol. **155**:3877.
4. Sarris, A.H. *et al.* (1993) J. Exp. Med. **178**:1127.
5. Angiolillo, A.L. *et al.* (1995) J. Exp. Med. **182**:155.
6. Neville, L.F. *et al.* (1997) Cytokine Growth Factor Rev. **8**:207.
7. Loetscher, M. *et al.* (1996) J. Exp. Med. **184**:963.
8. Weng, Y. *et al.* (1998) J. Biol. Chem. **273**:18288.
9. Jinquan, T. *et al.* (2000) J. Immunol. **165**:1548.
10. Jinquan, T. *et al.* (2000) Blood **96**:1230.
11. Hua, L.L. and S.C. Lee (2000) Glia **30**:74.
12. Kolb, S.A. *et al.* (1999) J. Neuroimmunol. **93**:172.
13. Kutsch, O. *et al.* (2000) J. Virol. **74**:9214.
14. Xia, M.Q. *et al.* (2000) J. Neuroimmunol. **108**:227.