

QUANTITATIVE DETERMINATION OF HUMAN IP-10

(INTERFERON-GAMMA INDUCIBLE PROTEIN 10KDA, CXCL10)

NEW PRODUCT

Human IP-10 ELISA

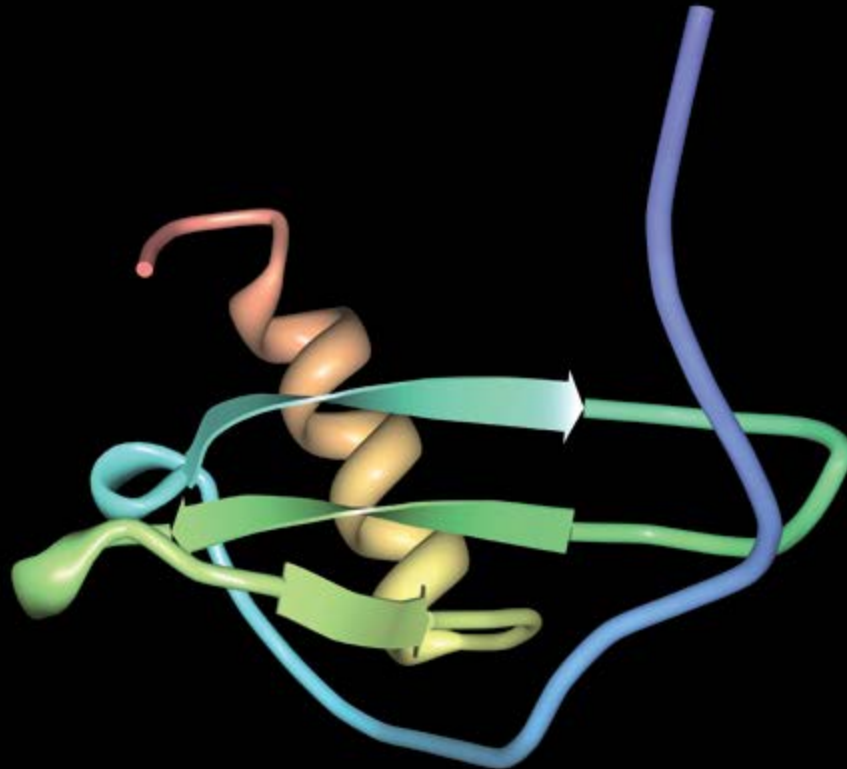
- › Prediction of response of PegIFN therapy in chronic hepatitis C and hepatitis B patients
- › High sensitivity (5.7 pg/ml)
- › Excellent analytical characteristics
- › Validated for human cell culture supernatant, serum and plasma samples



**IMMUNE RESPONSE, INFECTION
AND INFLAMMATION**

**CYTOKINES AND CHEMOKINES
AND RELATED MOLECULES**

HUMAN IP-10 ELISA



Introduction

IP-10 (Interferon-gamma inducible Protein 10kDa) also known as CXCL10, is secreted by several cell types in response to IFN-gamma and LPS. These cell types include monocytes, endothelial cells and fibroblasts [1]. 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 [1].

IP-10 has been attributed to several roles, 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 [2, 3].

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 [4].

IP-10 expression has been associated with HIV infection [5], is involved in inflammatory skin disease [6] 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) [7].

Pre-treatment IP-10 levels appear to predict response to peginterferon (PegIFN) therapy in chronic hepatitis C patients [8-11], independent of other known predictors, such as viral load, HCV genotype and stage of liver disease [8,10,11]. Peginterferon is also a first-line treatment option for chronic hepatitis B patients. Higher pre-treatment IP-10 levels are associated with an increased probability of HBeAg loss after PegIFN therapy. A combination of high baseline IP-10 and absence of precore (PC) and basal core promoter (BCP) mutants identified patients with the highest probability of combined response (HBeAg loss with HBV DNA <10,000 c/ml) and HBsAg loss [12].

QUANTITATIVE DETERMINATION OF HUMAN IP-10 (INTERFERON-GAMMA INDUCIBLE PROTEIN 10KDA, CXCL10)

BioVendor Human IP-10 ELISA (RGP019R)

Intended use

The RGP019R Human IP-10 ELISA kit is a solid phase sandwich ELISA for the in-vitro qualitative and quantitative determination of IP-10 (Interferon-gamma inducible Protein 10kDa) also known as CXCL10 in supernatants, buffered solutions or serum and plasma samples. This assay will recognise both natural and recombinant human IP-10.

- ▶ The total assay time is less than 4 hours
- ▶ The kit measures IP-10 protein in human cell culture supernatant, plasma and serum
- ▶ Assay format is 96 wells
- ▶ Components of the kit are provided ready to use, concentrated or lyophilized

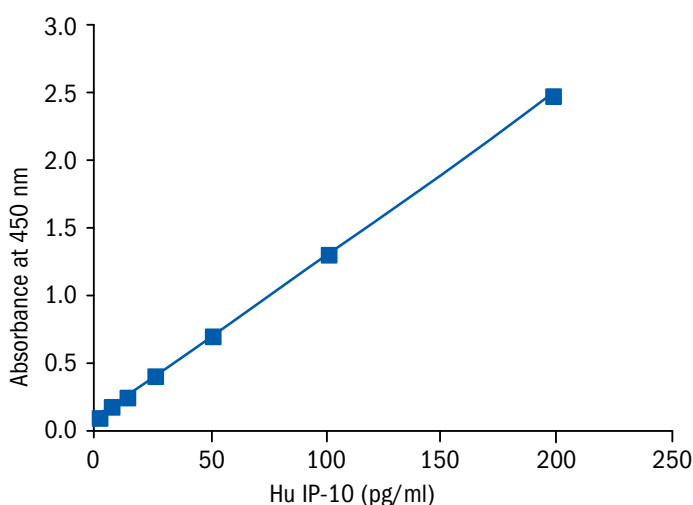
Clinical application

- ▶ Immune Response, Infection and Inflammation
- ▶ Cytokines and chemokines and related molecules

Test principle

A capture antibody highly specific for IP-10 has been coated to the wells of the microtitre strip plate provided during manufacture. 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 coloured complex with the conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of IP-10 present in the samples and standards. The absorbance of the colour 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.

HUMAN IP-10 ELISA CAT. NO.: RGP019R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Cell culture supernatant, Serum, Plasma
Standards	6.25 to 200 pg/ml
Limit of detection	5.7 pg/ml



Precision

Intra-assay (Within-Run) (n=6)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	60.1	0.9	1.6
2	32.4	0.5	1.5
3	19.6	0.3	1.3

Inter-assay (Run-to-Run) (n=18)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	56.9	2.7	4.7
2	29.9	2.0	6.5
3	17.7	1.5	8.5

Spiking recovery

The spiking recovery was evaluated by spiking different concentrations of recombinant IP-10 in human serum. Recoveries ranged from 118.7% to 122.4%.

Sensitivity

The sensitivity, minimum detectable dose of IP-10 using this BioVendor IP-10 ELISA kit 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.

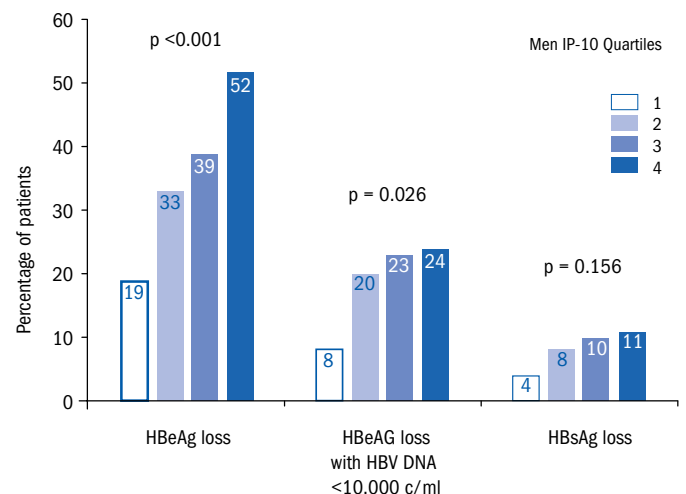
Summary of protocol

- Total procedure length: 3 h 45 min
- Add sample and diluted standard
- Incubate 2 hours at room temperature
- Wash plate 3 times
- Add 50 µl of biotinylated detection antibody
- Incubate 1 hour at room temperature
- Wash plate 3 times
- Add 100 µl of Streptavidin-HRP
- Incubate 30 min at room temperature
- Wash plate 3 times
- Add 100 µl of ready-to-use TMB
- Protect from light. Let the color develop for 10-20 min
- Add 100 µl H₂SO₄
- Read Absorbance at 450 nm

Clinical Relevance

Baseline IP-10 and response at 6 months post-treatment.

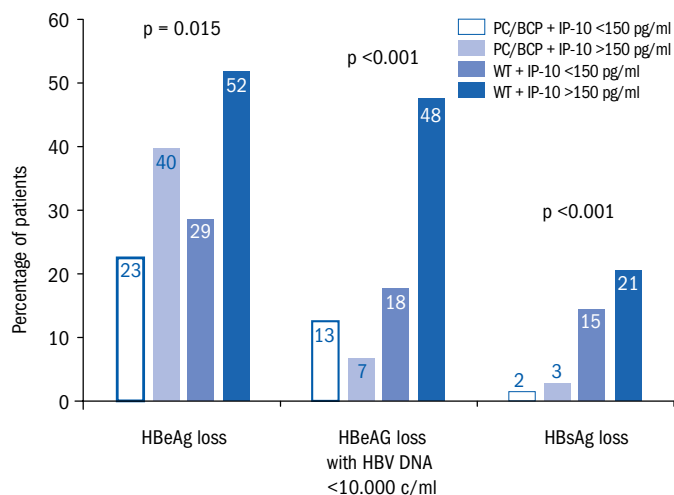
Relationship between baseline IP-10 and response to treatment in the HBeAg-positive population in the overall cohort.



HUMAN IP-10 ELISA

Observed response rates by baseline IP-10 and presence of PC and BCP mutants. The probability of combined response according to a baseline IP-10, in combination with the presence or absence of precore and/or core promoter mutants.

Source: Sonneveld MJ, Arends P, Boonstra A, Hansen BE, Janssen HL (2013): Serum levels of interferon-gamma-inducible protein 10 and response to peginterferon therapy in HBeAg-positive chronic hepatitis B. *J Hepatol.* 2013 May; 58(5):898-903.



PC = precore mutants, BCP = basal core promoter mutants

Related products

- RBMS6018R IP-10 Mouse ELISA
- RBG10196005 IP-10 Human *E. coli*
- RBG30062005 IP-10 Rat *E. coli*

References

1. Luster AD, Unkeless JC, Ravetch JV: Gamma-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. *Nature*. 1985 Jun 20;26;315(6021):672-6.
2. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, Luster AD: IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. *J Immunol*. 2002 Apr 1;168(7):3195-204.
3. Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S, Kleinman HK, Reaman GH, Tosato G: Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. *J Exp Med*. 1995 Jul 1;182(1):155-62.
4. Booth V, Keizer DW, Kamphuis MB, Clark-Lewis I, Sykes BD: The CXCR3 binding chemokine IP-10/CXCL10: structure and receptor interactions. *Biochemistry*. 2002 Aug 20;41(33):10418-25.
5. Kolb SA, Sporer B, Lahrz F, Koedel U, Pfister HW, Fontana A: Identification of a T cell chemotactic factor in the cerebrospinal fluid of HIV-1-infected individuals as interferon-gamma inducible protein 10. *J Neuroimmunol*. 1999 Jan 1;93(1-2):172-81.
6. Sebastiani S, Albanesi C, De PO, Puddu P, Cavani A, Girolomoni G: The role of chemokines in allergic contact dermatitis. *Arch Dermatol Res*. 2002 Jan;293(11):552-9.
7. Strieter RM, Polverini PJ, Arenberg DA, Kunkel SL: The role of CXC chemokines as regulators of angiogenesis. *Shock*. 1995 Sep;4(3):155-60.
8. Diago M, Castellano G, Garcia-Samaniego J, Perez C, Fernandez I, Romero M, et al.: Association of pretreatment serum interferon gamma inducible protein 10 levels with sustained virological response to peginterferon plus ribavirin therapy in genotype 1 infected patients with chronic hepatitis C. *Gut*. 2006;55:374-379.
9. Askarieh G, Alsio A, Pugnale P, Negro F, Ferrari C, Neumann AU, et al.: Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. *Hepatology*. 2010;51: 1523-1530.
10. Lagging M, Romero AI, Westin J, Norkrans G, Dhillon AP, Pawlotsky JM, et al.: IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection. *Hepatology*. 2006;44:1617-1625.
11. Romero AI, Lagging M, Westin J, Dhillon AP, Dustin LB, Pawlotsky JM, et al.: Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFNalpha 2a and ribavirin for chronic hepatitis C virus infection. *J Infect Dis*. 2006;194:895-903.
12. Sonneveld MJ, Arends P, Boonstra A, Hansen BA, Janssen HLA: Serum levels of interferon-gamma-inducible protein 10 and response to peginterferon therapy in HBeAg-positive chronic hepatitis B. *J Hepatol*. 2013 May;58(5):898-903.

Contact Information



BioVendor – Laboratorni medicina a.s.

Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420 549 124 185, Fax: +420 549 211 460
E-mail: info@biovendor.com

BioVendor GmbH

Otto-Hahn-Straße 16, 34123 Kassel, Germany
Phone: +49 6221 4339 100, Fax: +49 6221 4339 111
E-mail: infoEU@biovendor.com

› www.biovendor.com

BioVendor GesmbH

Gaudenzdorfer Gürtel 43-45, 1120 Vienna, Austria
Phone: +43 1 890 9025, Fax: +43 1 890 5163
E-mail: infoAustria@biovendor.com

BioVendor, LLC

128 Bingham Rd., Suite 1300, Asheville, NC 28806, USA
Phone: +1-800-404-7807, Phone: +1-828-575-9250
Fax: +1-828-575-9251, E-mail: infoUSA@biovendor.com