Quantikine® HS ELISA

Human IL-12 p70 Immunoassay

Catalog Number HS120
SS120
PHS120

For the quantitative determination of human Interleukin 12 p70 (IL-12 p70) 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 & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Interleukin 12 (IL-12; also NKSF) is a 70-75 kDa heterodimeric glycoprotein that belongs to the IL-12 family of heterodimeric cytokines (1-3). It consists of two disulfide-linked subunits which are 35 kDa (p35) and 40 kDa (p40) in size and share no meaningful amino acid (aa) sequence identity with each other (1, 4, 5). The mature p35 subunit is 197 aa in length and contains seven cysteines plus one potential N-linked glycosylation site (1-6). Mature human p35 shares 58% aa identity with mouse and rat p35 (2, 7, 8, 9). Mature human p40 is 306 aa in length, with 11 cysteines and three potential N-linked glycosylation sites, and it shares 66% aa identity with mouse and rat p40, respectively (1, 7, 10). While p35 resembles a hematopoietin ligand, p40 strongly resembles the N-terminus of a hematopoietin receptor, exhibiting a WSxWS motif, an immunoglobulin-like domain, and four conserved cysteines (1). This suggests that IL-12 may be a cytokine-receptor analog to the IL-6/soluble IL-6 R complex (4, 6). Notably, while p40 may circulate as either a monomer or homodimer, p35 is never found by itself (3). p40 does, however, serve as the larger of two subunits that comprise IL-23 (3, 11). Finally, while IL-12 is classically thought of as a secreted molecule, membrane-bound IL-12 has been reported on both human and mouse cells (12). Cells known to produce IL-12 include macrophages and dendritic cells (13), monocytes (14), Langerhans cells (15), neutrophils (16), keratinocytes (17), plasmacytoid dendritic cells (18), microglia (5), $CD8^+$ DC (mouse cells only) (19), and non-germinal center ($CD38^-CD44^+$) B cells (human cells only) (3, 20).

The high affinity receptor for human IL-12 is composed of at least two type I transmembrane glycoproteins that resemble members of the cytokine receptor superfamily. The first subunit (Rβ1) is 100 kDa in size and binds IL-12 with a Kd=1 nM (21). This receptor serves as the principal binding site for the p40 subunit (4, 5). The second subunit (Rβ2) is 130 kDa in size and shows no meaningful aa sequence identity to the Rβ1 subunit (5, 21, 22). This receptor appears to be the principal signal transduction component and is suggested to serve as an attachment point for a disulfide-linked p35-p40 heterodimer (4, 5, 22). As noted above, human p40 will circulate either as a monomer, homodimer, or in a complex bound to either p35, forming IL-12, or to p19, forming IL-23 (3-5, 11). Both the homodimeric p40, and IL-23 can bind to the IL-12 R, serving as nonsignaling antagonists (3, 23, 24). Alternatively, the p40 homodimer may also bind to Rβ1, activating microglia and macrophages (4, 25).

Functionally, IL-12 has been shown to both enhance cytotoxic activity and induce interferon-gamma (IFN-γ) production in NK cells, T cells and dendritic epidermal T cells (3, 26, 27, 28). IL-12 has also been reported to induce IFN-γ production in macrophages (29). IL-12, in conjunction with the other IL-12 family members IL-23 and IL-27, promotes the development of a $CD4^+$ Th1 immune response (4, 5, 30). In response to infection, IL-27 is released initially, promoting a Th0 to Th0/1 transition. IL-12 production follows, generating Th1 effector cells. In combination with IL-18, IL-12 creates Th1 memory cells out of effector cells, and these cells are later activated by IL-23 (4).

The Quantikine HS Human IL-12 p70 Immunoassay is a 6.5 hour solid phase ELISA designed to measure IL-12 p70 in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human IL-12 p70 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human IL-12 p70 accurately. Results obtained using natural human IL-12 p70 showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-12 p70.

PRINCIPLE OF THE ASSAY

DUE TO THE HIGH SENSITIVITY OF THIS KIT, PLEASE NOTE THE FOLLOWING PRECAUTIONS.

- **Alkaline phosphatase is detectable in saliva.** Take precautionary measures (*e.g.* wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).
- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize nonspecific binding of the conjugate.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-12 p70 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-12 p70 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-12 p70 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells and color develops in proportion to the amount of IL-12 p70 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.
- Although this kit has been designed to eliminate matrix problems, there may exist some samples that give falsely elevated values when assayed neat. If samples generate values that are suspiciously high or higher than the highest standard, dilute the samples in the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard 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

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing 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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Neither the addition of the Substrate Solution nor the Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the plate in the same order as 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 # HS120	CATALOG # SS120	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
IL-12 p70 Microplate	890398	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human IL-12 p70.	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.*
IL-12 p70 Standard	890257	1 vial	6 vials	200 pg/vial of recombinant human IL-12 p70 in a buffered protein base with preservatives; lyophilized.	May be stored for up to 1 month at 2-8 °C.*
IL-12 p70 Conjugate	890259	1 vial	6 vials	21 mL/vial of polyclonal antibody against human IL-12 p70 conjugated to alkaline phosphatase with preservatives.	
Assay Diluent HD1-7	895160	1 vial	6 vials	6 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD5R	895190	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Assay Diluent RD1-5	895608	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *Contains a precipitate. Mix well before and during use. For serum/plasma samples.*	
Calibrator Diluent RD6P	895118	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895188	1 vial	6 vials	100 mL/vial of a 10-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Substrate	895884	1 vial	6 vials	Lyophilized NADPH with stabilizers.	**Store reconstituted substrate and amplifier solutions in an upright position** for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Substrate Diluent	895885	1 vial	6 vials	7 mL/vial of buffered solution with stabilizers.	
Amplifier	895886	1 vial	6 vials	Lyophilized amplifier enzymes with stabilizers.	
Amplifier Diluent	895887	1 vial	6 vials	7 mL/vial of buffered solution containing INT-violet with stabilizer.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

HS120 contains sufficient materials to run an ELISA on one 96 well plate.
SS120 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PHS120). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm, with dual wavelength correction set at 650 nm or 690 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 1000 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human IL-12 p70 Controls (optional; available from R&D Systems).

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Alkaline phosphatase is detectable in saliva. Take precautionary measures (*e.g.* wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).

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

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

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS 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 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 is not recommended for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

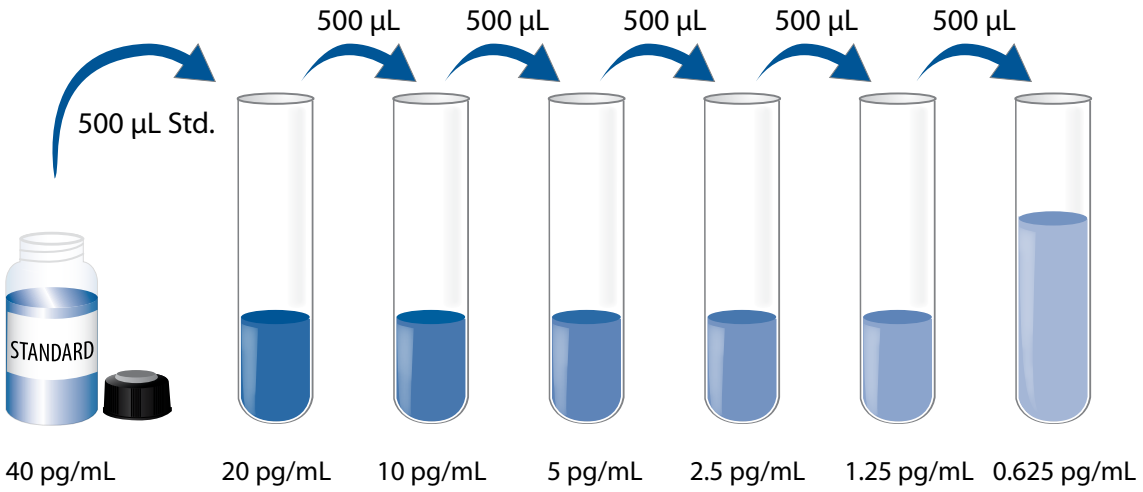
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Reconstitute the lyophilized Substrate in 6 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier in 6 mL of Amplifier Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

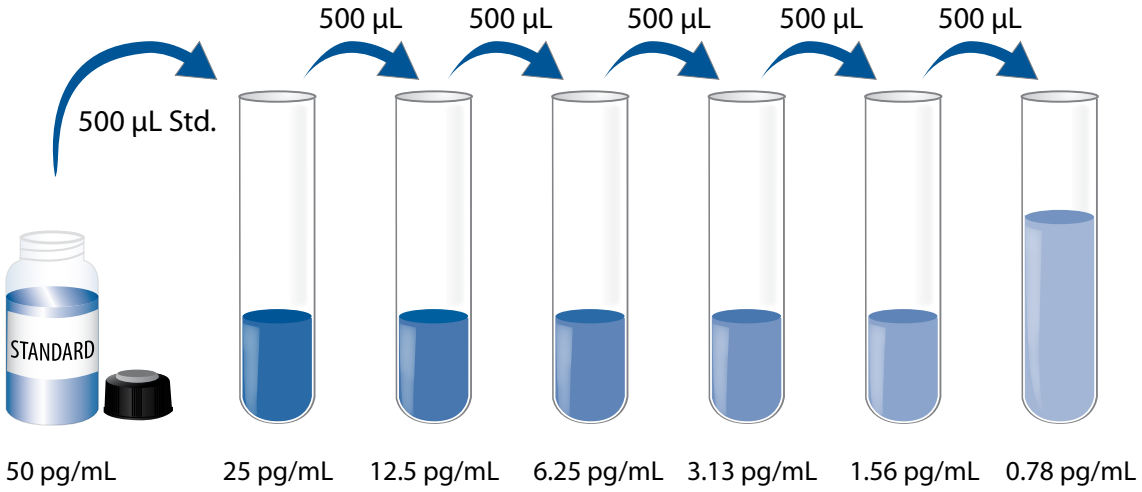
Cell Culture Supernate Standard - Reconstitute the IL-12 p70 Standard with 5.0 mL of Calibrator Diluent RD5R. This reconstitution produces a stock solution of 40 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD5R into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted IL-12 p70 Standard (40 pg/mL) serves as the high standard. Calibrator Diluent RD5R serves as the zero standard (0 pg/mL).



Serum/Plasma Standard - Reconstitute the IL-12 p70 Standard with 4 mL of Calibrator Diluent RD6P. This reconstitution produces a stock solution of 50 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD6P into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted IL-12 p70 Standard (50 pg/mL) serves as the high standard. Calibrator Diluent RD6P 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, samples, and controls be assayed in duplicate.

Note: *Alkaline phosphatase is detectable in saliva. Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

1. Prepare all reagents 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 Assay Diluent to each well.
   For Cell Culture Supernate Samples: Add 50 µL of Assay Diluent HD1-7 to each well.
   For Serum/Plasma Samples: Add 50 µL of Assay Diluent RD1-5 to each well. *Assay Diluent RD1-5 contains a precipitate. Mix well before and during use.*
4. Add 200 µL of Standard or sample per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Wash.

Notes on washing

- *Excessive drying of the wells can lead to poor assay performance and imprecision. Subsequent reagents should be added immediately after washing the plate, and the wells not allowed to completely dry. Also avoid prolonged exposure of the wells to vacuum aspiration apparatus.*
- *Inclusion of a 30 second soak between each addition of Wash Buffer and decanting of the plate contents will improve the precision of the assay.*

Wash Procedure

a. Remove liquid from the wells by inverting the plate and decanting the contents.
b. Remove excess liquid by grasping the plate firmly and smartly rapping the inverted plate on a clean paper towel at least 5 times.
c. Fill each well with 400 µL of Wash Buffer using an autowasher, squirt bottle, or a manifold dispenser.
d. Remove liquid from the wells by inverting the plate and decanting the contents.
e. Repeat steps b-d 5 times for a total of 6 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.

6. Add 200 µL of IL-12 p70 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the wash as in step 5.
8. Add 50 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature. **Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature. **Note:** *Addition of Amplifier Solution initiates color development.*
10. Add 50 µL of Stop Solution to each well. Addition of Stop Solution does not affect the color in the wells.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set at 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 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.).

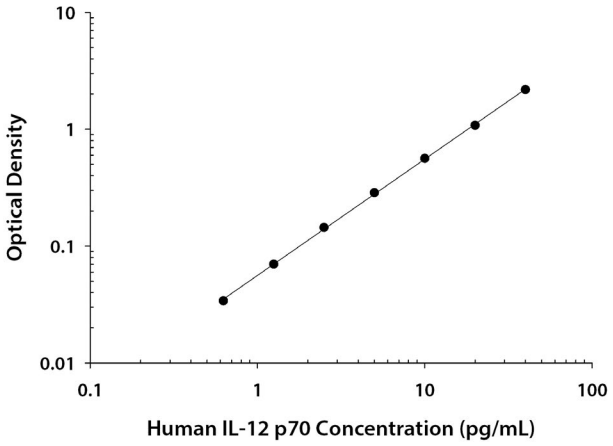
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-12 p70 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

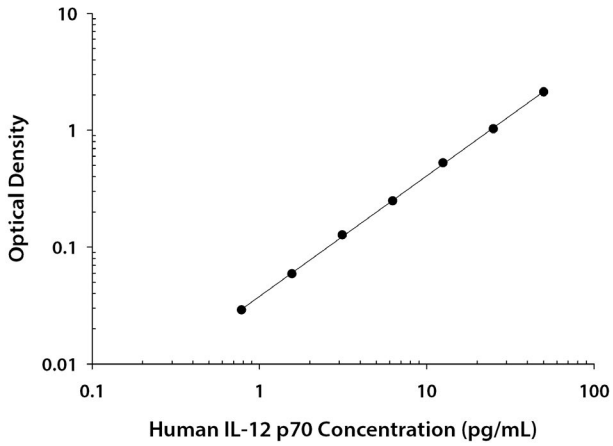
The following standard curves are provided for demonstration only. A standard curve must be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.131 0.136	0.134	—
0.625	0.166 0.169	0.168	0.034
1.25	0.204 0.204	0.204	0.070
2.5	0.274 0.283	0.278	0.144
5	0.418 0.422	0.420	0.286
10	0.690 0.701	0.696	0.562
20	1.175 1.248	1.212	1.078
40	2.297 2.321	2.309	2.175

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.092 0.094	0.093	—
0.78	0.122 0.122	0.122	0.029
1.56	0.151 0.154	0.152	0.059
3.13	0.219 0.221	0.220	0.127
6.25	0.337 0.348	0.342	0.249
12.5	0.618 0.623	0.620	0.527
25	1.075 1.163	1.119	1.026
50	2.207 2.233	2.220	2.127

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 twenty separate assays to assess inter-assay precision.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	5.32	13.0	25.0	2.86	8.49	29.6
Standard deviation	0.24	0.71	1.89	0.5	0.85	2.43
CV (%)	4.5	5.5	7.6	17.5	10.0	8.2

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	5.26	18.6	35.8	2.86	9.25	36.4
Standard deviation	0.26	0.77	0.90	0.36	1.39	2.76
CV (%)	4.9	4.1	2.5	12.6	15.0	7.6

RECOVERY

The recovery of human IL-12 p70 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	95	85-107%
Serum (n=5)	100	91-115%
EDTA plasma (n=5)	102	83-117%
Heparin plasma (n=5)	97	86-111%

SENSITIVITY

The minimum detectable dose (MDD) of human IL-12 p70 is typically less than 0.5 pg/mL.

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

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IL-12 p70 in various matrices and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	99	102	100	98
	Range (%)	92-103	100-104	98-105	85-106
1:4	Average % of Expected	102	109	98	103
	Range (%)	97-107	108-112	94-103	86-112
1:8	Average % of Expected	106	108	100	103
	Range (%)	97-115	100-114	90-113	93-109
1:16	Average % of Expected	109	105	97	97
	Range (%)	96-135	90-113	89-113	82-113

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human IL-12 p70 produced at R&D Systems.

The NIBSC/WHO recombinant human IL-12 Reference Reagent 95/544 was evaluated in this kit. The dose response curve of the NIBSC Standard parallels the Quantikine HS standard curve. To convert sample values obtained with the Quantikine HS Human IL-12 p70 kit to approximate NIBSC 95/544 Units, use the equation below:

NIBSC/WHO (95/544) approximate value (U/mL) = 0.016 x Quantikine HS Human IL-12 p70 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of IL-12 p70 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=45)	1.93	18	ND-3.58
EDTA plasma (n=45)	1.86	20	ND-3.78
Heparin plasma (n=45)	1.55	18	ND-2.80

ND=Non-detectable

Cell Culture Supernates - NC-37 human Burkitt's lymphoma B lymphoblast cells (1 x 10^6 cells/mL) were cultured for 3 days in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, 1% Nutridoma, and 25 ng/mL calcium ionophore, and stimulated with 10 ng/mL PMA. The cell culture supernate was assayed for human IL-12 p70 and measured 30.4 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-12 p70.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range human IL-12 p70 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

G-CSF
IL-6
IL-6 Rα
IL-12/IL-23 p40
IL-12 Rβ1
IL-12 Rβ2
IL-27

Recombinant mouse:

IL-6
IL-12 p70
IL-12/IL-23 p40
IL-23
IL-27

Recombinant human IL-23 cross-reacts approximately 0.9% in this assay.

REFERENCES

1. Schoenhaut, D.S. *et al.* (1992) J. Immunol. **148**:3433.
2. Stern, A.S. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:6808.
3. Hamza, T. *et al.* (2010) Int. J. Mol. Sci. **11**:789.
4. Brombacher, F. *et al.* (2003) Trends Immunol. **24**:207.
5. Trinchieri, G. (2003) Nat. Rev. Immunol. **3**:133.
6. Gearing, D.P. and D. Cosman (1991) Cell **66**:9.
7. Gubler, U. *et al.* (1991) Proc. Natl. Acad. Sci. USA **88**:4143.
8. Wolf, S.F. *et al.* (1991) J. Immunol. **146**:3074.
9. Verma, N.D. *et al.* (2004) SwissProt Accession #:Q9R103.
10. Khalife, J. *et al.* (1998) Eur. Cytokine Netw. **9**:69.
11. Gee, K. *et al.* (2009) Inflamm. Allergy Drug Targets **8**:40.
12. Fan, X. *et al.* (1996) Biochem. Biophys. Res. Commun. **225**:1063.
13. Kato, T. *et al.* (1997) Cell. Immunol. **181**:59.
14. Blotta, M.H. *et al.* (1997) J. Immunol. **158**:5589.
15. Kang, K. *et al.* (1996) J. Immunol. **156**:1402.
16. Romani, L. *et al.* (1997) J. Immunol. **158**:5349.
17. Yawalkar, N. *et al.* (1996) J. Invest. Dermatol. **106**:80.
18. Krug, A. *et al.* (2001) Eur. J. Immunol. **31**:3026.
19. Shortman, K. and W. Heath (2010) Immunol. Rev. **234**:18.
20. Schultze, J.L. *et al.* (1999) J. Exp. Med. **189**:1.
21. Chua, A.O. *et al.* (1994) J. Immunol. **153**:128.
22. Presky, D.H. *et al.* (1996) Proc. Natl. Acad. Sci. USA **93**:14002.
23. Heinzel, F.P. *et al.* (1997) J. Immunol. **158**:4381.
24. Sieve, A.N. *et al.* (2010) Eur. J. Immunol. **40**:2236.
25. Jana, M. *et al.* (2009) Glia **57**:1553.
26. Novelli, F. and J.L. Casanova (2004) Cytokine Growth Factor Rev. **15**:367.
27. Sugaya, M. *et al.* (1999) J. Invest. Dermatol. **113**:350.
28. Tominaga, K. *et al.* (2000) Int. Immunol. **12**:151.
29. Pudda, P. *et al.* (1997) J. Immunol. **159**:3490.
30. Collison, L.W. and D. Vignali (2008) Immunol. Rev. **226**:248.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.