ELISA Kit

Catalog # KHC0121: (96 tests)
KHC0122: (192 tests)

Human
IL-12 p40/p70

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TABLE OF CONTENTS

INTRODUCTION

Human Interleukin-12 (Hu IL-12) is a 75 kDa lymphokine produced mainly by monocytes, macrophages, B-lymphocytes and dendritic cells. IL-12 shows an unusual heterodimeric structure composed of one 40 kDa (p40) and one 35 kDa (p35) subunit linked together by disulfide bonds. The p35 subunit is distantly related to IL-6 and G-CSF while the p40 subunit shows homology to the extracellular domain of the IL-6 receptor α chain. This suggests that IL-12 may have evolved from a cytokine/soluble receptor complex. p40 is secreted in large excess over the biologically active heterodimer. p40 is involved in receptor binding but p35 is necessary for signal transduction. Monomers and homodimers of p40 show antagonist activity to IL-12. *In vivo*, IL-12 appears to play a major role in auto-immune disease, resistance to bacterial and parasitic infections, antiviral responses including HIV, and the promotion of anti-tumor immunity. IL-12 has been shown to be a powerful adjuvent in vaccination.

PURPOSE

The Invitrogen Human IL-12 p40/p70 ELISA is to be used for the quantitative determination of Hu IL-12 in human serum, plasma, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant forms of the IL-12 heterodimer as well as the free p40 subunit.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen human IL-12 p40/p70 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu IL-12 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu IL-12 content, control specimens, and unknowns are pipetted into these wells followed by the addition of a second biotinylated monoclonal antibody.

During the first incubation, the Hu IL-12 antigen binds to the immobilized (capture) antibody on one site and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu IL-12 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***	***192 Test Kit***	***480 Test Kit***
Hu IL-12 Standard, purified recombinant Hu *IL-12* expressed in *E. coli*. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	10 vials
Standard Diluent Buffer. Contains 8 mM sodium azide; 25 mL per bottle.	1 bottle	2 bottles	5 bottles
Hu IL-12 Antibody-Coated Wells, 96 wells per plate.	1 plate	2 plates	5 plates
Hu IL-12 Biotin Conjugate, (Biotin-labeled anti-*IL-12 p40*). Contains 8 mM sodium azide; 11 mL per bottle.	1 bottle	2 bottles	5 bottles
Streptavidin-Peroxidase (HRP), concentrate (100x); Contains 0.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials	5 vials
Streptavidin-Peroxidase (HRP) Diluent. Contains 3 mM thymol and 0.05% Proclin® 300; 25 mL per bottle.	1 bottle	1 bottle	3 bottles
Wash Buffer Concentrate (25x); 100 mL per bottle.	1 bottle	1 bottle	2 bottles
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle	1 bottle	3 bottles
Stop Solution; 25 mL per bottle.	1 bottle	1 bottle	3 bottles
Plate Covers, adhesive strips.	3	4	15

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Proclin® 300 is toxic. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are >500 pg/mL should be diluted with *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. 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.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of Hu IL-12 Standard

This assay has been calibrated against the International Standard preparation (95/544) for Hu IL-12 (NIBSC, Hertfordshire, UK, EN6 3QG). One picogram of standard is equivalent to 22 mIU of NIBSC (95/544).

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 5000 pg/mL with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.100 mL of the reconstituted standard to a tube containing 0.900 mL *Standard Diluent Buffer*. Label as 500 pg/mL Hu IL-12. Mix.
3. Add 0.200 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 250, 125, 62.5, 31.2, 15.6, and 7.8 pg/mL Hu IL-12.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Hu IL-12 Standard

Standard:	Add:	Into:
500 pg/mL	Prepare as described in Step 2.	
250 pg/mL	0.200 mL of the 500 pg/mL std.	0.200 mL of the Diluent Buffer
125 pg/mL	0.200mL of the 250 pg/mL std.	0.200 mL of the Diluent Buffer
62.5 pg/mL	0.200 mL of the 125 pg/mL std.	0.200 mL of the Diluent Buffer
31.2 pg/mL	0.200 mL of the 62.5 pg/mL std.	0.200 mL of the Diluent Buffer
15.6 pg/mL	0.200 mL of the 31.2 pg/mL std.	0.200 mL of the Diluent Buffer
7.8 pg/mL	0.200 mL of the 15.6 pg/mL std.	0.200 mL of the Diluent Buffer
0 pg/mL	0.200 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing the assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

D. Dilution of Wash Buffer

Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 50 µL of standards, samples or controls to the appropriate microtiter wells. (See **REAGENT PREPARATION AND STORAGE**, Section B.)
4. Pipette 100 µL of biotinylated anti-IL-12 (*Biotin Conjugate*) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
5. Cover plate with a *plate cover* and incubate for **2 hours at room temperature**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**

7. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
8. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature.**
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**
10. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
12. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution.*

14. Plot on graph paper absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from all *data* points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
15. Read the Hu IL-12 concentrations for unknown samples and controls from the standard curve plotted in Step 14. Samples producing signals greater than that of the highest standard (500 pg/mL) should be diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 500 pg/mL Hu IL-12.

Standard Hu IL-12 (pg/mL)	Optical Density (450 nm)
0	0.054
	0.059
7.8	0.126
	0.127
15.6	0.203
	0.191
31.2	0.345
	0.323
62.5	0.611
	0.600
125	1.210
	1.081
250	2.042
	2.062
500	3.528
	3.465

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 500 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >500 pg/mL with *Standard Diluent Buffer*, reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu IL-12 p40/p70 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Hu IL-12 p40/p70 is <2.0 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 24 times.

PRECISION

1. Intra-Assay Precision

Samples of known Hu IL-12 p40/p70 concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	79.7	189.0	384.1
SD	3.1	7.3	7.1
%CV	3.9	3.9	1.8

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 40 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	79.3	187.6	370.2
SD	3.1	5.5	14.8
%CV	3.9	2.9	4.0

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Culture medium containing 412 pg/mL of measured Hu IL-12 p40/p70 was serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell Culture		
	Measured (pg/mL)	**Expected (pg/mL)**	**% Expected**
neat	**412**	**-**	**-**
1/2	**191.7**	**206**	**93.1**
1/4	**95**	**103**	**92.2**
1/8	**52.6**	**51.5**	**102.1**
1/16	**25.3**	**25.7**	**98.4**
1/32	**12.4**	**12.8**	**96.9**

RECOVERY

The recovery of Hu IL-12 p40/p70 added to human serum, EDTA plasma and heparinized plasma averaged 97%, 94%, and 90%, respectively. The recovery of Hu IL-12 p40/p70 added to tissue culture medium containing 1% and 10% fetal bovine serum averaged 105% and 116%, respectively.

EXPECTED VALUES

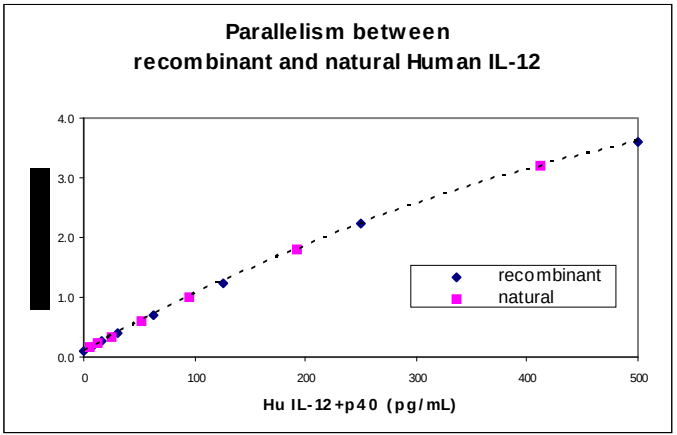
Each laboratory must establish its own normal values. For guidance, the mean of 12 normal sera was 74.9 pg/mL (SD=28.9), ranging between 40.4 and 150 pg/mL.

The mean of 12 normal EDTA plasma samples was 67.8 pg/mL (SD=27.5) ranging between 37.4 and 140.9 pg/mL.

The mean of 12 normal heparinized plasma samples was 55.8 pg/mL (SD= 26.9) ranging between 31.8 and 131 pg/mL.

PARALLELISM

Natural Hu IL-12 p40/p70 was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant protein was demonstrated by the figure below and indicated that the standard accurately reflects natural Hu IL-12 content in samples.



SPECIFICITY

Buffered solutions of a panel of substances at 150 ng/mL were assayed with the Invitrogen Hu IL-12 p40/p70 kit. The following substances were tested and found to have no cross-reactivity: human IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-15, IFN-γ, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, GRO, IP-10, TNF-α, TNF-β, MCP-3, RANTES, NAP-2, SCF, PDGF.

REFERENCES

1. Brunda, M.J. (1994) Interleukin-12. *Journal of Leukocyte Biology* 55:280-288.
2. Trinchieri, G. (1993) Interleukin-12 and its role in the generation of Th1 cells. *Immunology Today* 14(7):335-337.
3. Trinchieri, G. et al. (1994) The role of Interleukin-12 in the immune response, disease and therapy. *Immunology Today* 15-10:460-463.
4. Zhang, M. (1994) Interleukin-12 at the site of disease in tuberculosis. *J. Clin. Invest*. 93:1733-1739.
5. Podlaski, F.J. et al. (1992) Molecular characterization of Interleukin-12. *Arch. of Biochem. Biophy*. 294-1:230-237.

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

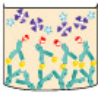
Human IL-12 p40/p70 Assay Summary

Add 50 µL of standards, controls & samples

Add 100 µL of Biotin Conjugate
Incubate for 2 hours at RT

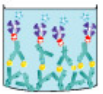
aspirate and wash 4x

Incubate 100 µL of Streptavidin-HRP
Working Solution for 30 minutes at RT

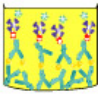


aspirate and wash 4x

Incubate 100 µL of Stabilized Chromogen
for 30 minutes at RT



Add 100 µL of Stop Solution
and read at 450 nm



Total time: 3 hours