Human IL-12 p70 ELISA Kit

Catalog Number KAC1568 (96 tests)

Pub. No. MAN0018669 **Rev.** A.0

CAUTION! This kit contains materials with small quantities of sodium azide and Proclin™ 300. 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. In case of contact, rinse affected area with plenty of water. Proclin™ 300 is toxic, corrosive, and a skin irritant. 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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human IL-12 p70 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human IL-12 p70 in human serum, plasma, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant human IL-12 p70.

Human IL-12 is a 70 kDa (p70) 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. p35 subunit is distantly related to IL-6 and G-CSF while p40 shows homology to the extracellular domain of the α chain of the IL-6 receptor.

Contents and Storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KAC1568 (96 tests)
Hu IL-12 p70 Standard; lyophilized. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer; contains 8 mM sodium azide, 0.05% Proclin™ 300, 0.05% thymol, and 0.1% benzamidine	25 mL
Hu IL-12 p70 Antibody-Coated Wells, 96-well strip-well plate	1 plate
Hu IL-12 p70 Biotin Conjugate; contains 8 mM sodium azide	6 mL
Streptavidin-Peroxidase (HRP) (100X); contains 3.3 mM thymol	0.15 mL
Streptavidin-Peroxidase (HRP) Diluent; contains 0.05% Proclin™ 300	25 mL
Incubation Buffer, contains 8 mM sodium azide	11 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

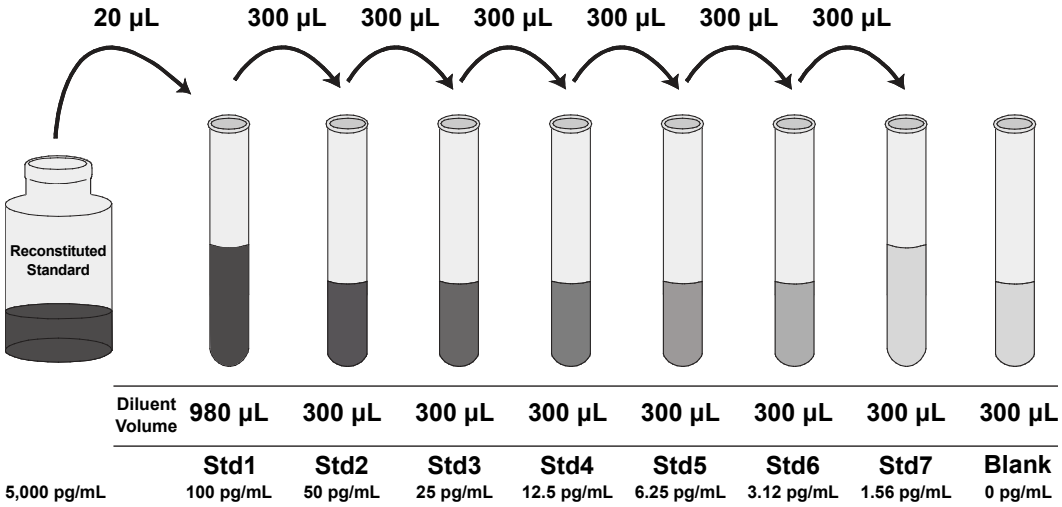
- Dilute samples with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This assay has been calibrated against the WHO reference preparation (NIBSC, Hertfordshire, UK, EN6 3QG). One picogram of standard equals 10 mIU of NIBSC standard (95/544).

1. Reconstitute Hu IL-12 p70 Standard to 5,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 5,000 pg/mL human IL-12 p70. Use the standard within **1 hour** of reconstitution.
2. Add 20 µL reconstituted standard to one tube containing 980 µL Standard Diluent Buffer and mix. Label as 100 pg/mL human IL-12 p70.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.12, 1.56 and 0 pg/mL human IL-12 p70.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

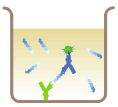
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

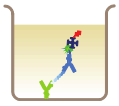
- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 50 µL of **Incubation Buffer** to wells containing serum/plama samples, standards, and controls; or 50 µL of **Standard Diluent Buffer** to the wells containing buffered solutions or cell culture samples. Leave the wells for chromogen blanks empty.
b. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells.
c. Add 50 µL Hu IL-12 p70 Biotin Conjugate solution into each well except the chromogen blanks.
d. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 3 hours at room temperature.
e. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Streptavidin-HRP



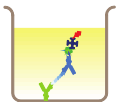
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

4 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 100 pg/mL human IL-12 p70.

Standard Human IL-12 p70 (pg/mL)	Optical Density (450 nm)
100	2.88
50	1.88
25	1.13
12.5	0.65
6.25	0.43
3.12	0.29
1.56	0.19
0	0.11

Sensitivity

The analytical sensitivity of human IL-12 p70 is 0.2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 24 times.

Inter-assay precision

Samples were assayed 16 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	3.8	11.9	44.4
Standard Deviation	0.31	0.87	3.64
% Coefficient of Variation	8.2	7.3	8.2

Intra-assay precision

Samples of known human IL-12 p70 concentration were assayed in replicates of 24 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	4.5	13.8	48.3
Standard Deviation	0.34	0.89	3.82
% Coefficient of Variation	7.6	6.4	7.9

Expected values

Forty serum and EDTA plasma samples were evaluated in the assay.

Sample	Range (pg/mL)	Avg of Detectable[1] (pg/mL)
Serum (n=20)	ND[2]–0.79	0.47
EDTA plasma (n=20)	ND[2]–0.62	0.47

[1] Detectable: >0.2 pg/mL

[2] Not detected

Human monocytic cells (THP-1) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate. Cells were pre-treated for 6 days with 75 ng/mL of GM-CSF, then stimulated with:

- 1 µg/mL of LPS
- 1 µg/mL of LPS + 1 µg/mL IFN-γ
- Log-phase *E. coli* (50 µL/6 mL culture medium)
- Log-phase *E. coli* (50 µL/6 mL culture medium) + 1 µg/mL IFN-γ

The supernatants were collected at 24 hours and measured.

Cell Type	Stimulus	IL-12 (pg/mL)
THP-1	Neat	ND[1]
THP-1	LPS	ND[1]
THP-1	LPS + IFN-γ	41
THP-1	*E. coli*	ND[1]
THP-1	*E. coli* + IFN-γ	170

[1] Not detected

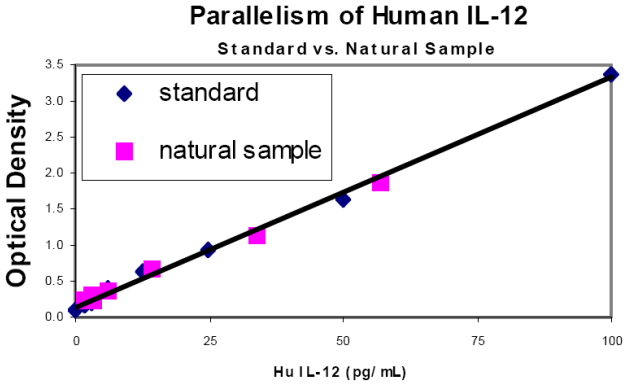
Linearity of dilution

Human serum or cell culture samples containing human IL-12 p70 were serially diluted over the range of the assay in Standard Diluent Buffer or RPMI containing 10% fetal calf serum, respectively. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.99.

Dilution	Serum			Cell Culture		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
1/1	72.7	—	—	64.6	—	—
1/2	35.5	36.3	98	34.7	32.3	107
1/4	18.1	18.2	100	15.8	16.2	98
1/8	8.8	9.1	97	8.4	8.1	104
1/16	4.9	4.5	108	3.8	4.0	94

Parallelism

Natural human IL-12 p70 was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects the full human IL-12 p70 content in samples.



Recovery

Recovery of human IL-12 p70 in various samples. Low recoveries were observed in some serum/plasma samples, but addition of Pefabloc at a final concentration of 1 mg/mL in these samples restored 90 to 100% recoveries. Pefabloc is a serine protease inhibitor supplied by Roche (Cat. # 11 429 868 001).

Sample	Average % Recovery
Serum	87
EDTA plasma	99
Human heparinized plasma	97
Human citrated plasma	95
Cell culture medium + 1% fetal calf serum	98
Cell culture medium + 10% fetal calf serum	102

Specificity

Buffered solutions of a panel of substances at 150 ng/mL were assayed with the kit. The following substances were tested and found to have no cross-reactivity: **human** IFN-γ, TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-16, IP-10, GRO, MCAF, MCP3, MIP-1α, SCF, Oncostatin-M, LIF, Leptin. Human IL-23 and the p40 subunit of Hu IL-12 were found to have no significant cross-reactivity (<0.01%) and did not interfere with the quantitation of human IL-12 p70.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.