Human IL-12 (p70) ELISA Kit

EZHIL12

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

IL-12 (p70) is a potent regulator of cell-mediated immune responses that induces IFN-γ production by NK and T cells. It is produced by activated monocytes / macrophages, B lymphocytes, and connective tissue type mast cells as a 70 kD heterodimeric glycoprotein comprised of disulfide-bonded 35 kD and 40 kD subunits. Among its biological activities, IL-12 promotes the growth and activity of activated NK, CD4+, and CD8+ cells and induces the development of IFN-γ producing Th1 cells.

The Human IL-12 (p70) ELISA Kit is a Sandwich Enzyme- Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody.

This kit is specifically designed for the accurate quantification of human IL-12 (p70) from cell culture supernatant, serum, plasma and other biological fluids. This kit is analytically validated with ready-to-use reagents.

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Materials Provided

Store at 2℃ to 8℃		
Component	**Item No.**	**Volume**
Anti-Human IL-12 (p70) pre-coated 96-well plate	CS211394	1 plate
Human IL-12 (p70) Detection Antibody	CS211395	1 bottle (12 mL)
Human IL-12 (p70) Standard	CS211396	1 vial (lyophilized)
Avidin-HRP A	CS210051	1 bottle (12 mL)
Assay Buffer A	CS210062	1 bottle (25 mL)
Matrix A (for serum and plasma samples only)	CS210066	1 vial (lyophilized)
Wash Buffer (20X)	CS210053	1 bottle (50 mL)
Substrate Solution F	CS210054	1 bottle (12 mL)
Stop Solution	CS213422	1 bottle (12 mL)
Plate Sealers	CS210056	4 sheets

Materials Required But Not Supplied

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1 mL
- Deionized water
- Wash bottle or automated microplate washer
- Log-log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper
- Plate Shaker

Warnings and Precautions

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and plasma in accordance with NCCLS regulations.
- Stop Solution contains acid. *Wear eye, hand, and face protection.*
- Before disposing the plate at the end, rinse it with an excess amount of water.

Storage and Stability

Store at 2℃-8ºC; performance guaranteed for 4 months from date of receipt when reagents are stored properly.

Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at -70℃. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70℃. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70℃. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.
2. Reconstitute the lyophilized Human IL-12 (p70) Standard by adding the volume of Assay Buffer A indicated on the vial label to make the 20 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely. Store any unused 20 ng/mL standard stock solution at -70℃ in aliquots in polypropylene vials to prevent repeated freeze-thaw cycles.
3. If serum or plasma samples will be assayed, reconstitute the lyophilized Matrix A by dispensing 2 mL of deionized water into the vial and allow the reconstituted Matrix A to sit at room temperature for 15 minutes; vortex to mix completely.
4. In general, samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer A as the diluent for cell culture supernatant and Matrix A as the diluent for serum or plasma samples.

Protocol

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 500 µL of the 500 pg/mL top standard by diluting 12.5 µL of the standard stock solution in 487.5 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 500 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human IL-12 (p70) standard concentrations in the tubes are 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, and 7.8 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).

4. Wash plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. For measuring samples of cell culture supernatant:

a) Add 50 µL of Assay Buffer A to each of the wells that will contain either standard dilutions or samples.

b) Add 50 µL of each standard dilution or sample to an appropriate well.

For measuring serum or plasma samples:

a) Add 50 µL of Matrix A to each of the wells that will contain the standard dilutions. Add 50 µL of Assay Buffer A to each of the wells that will contain samples

b) Add 50 µL of standard dilutions to each of the wells containing Matrix A. Add 50 µL of serum or plasma samples to each of the wells containing Assay Buffer A.

6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.

7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

8. Add 100 µL of Human IL-12 (p70) Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

10 . Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

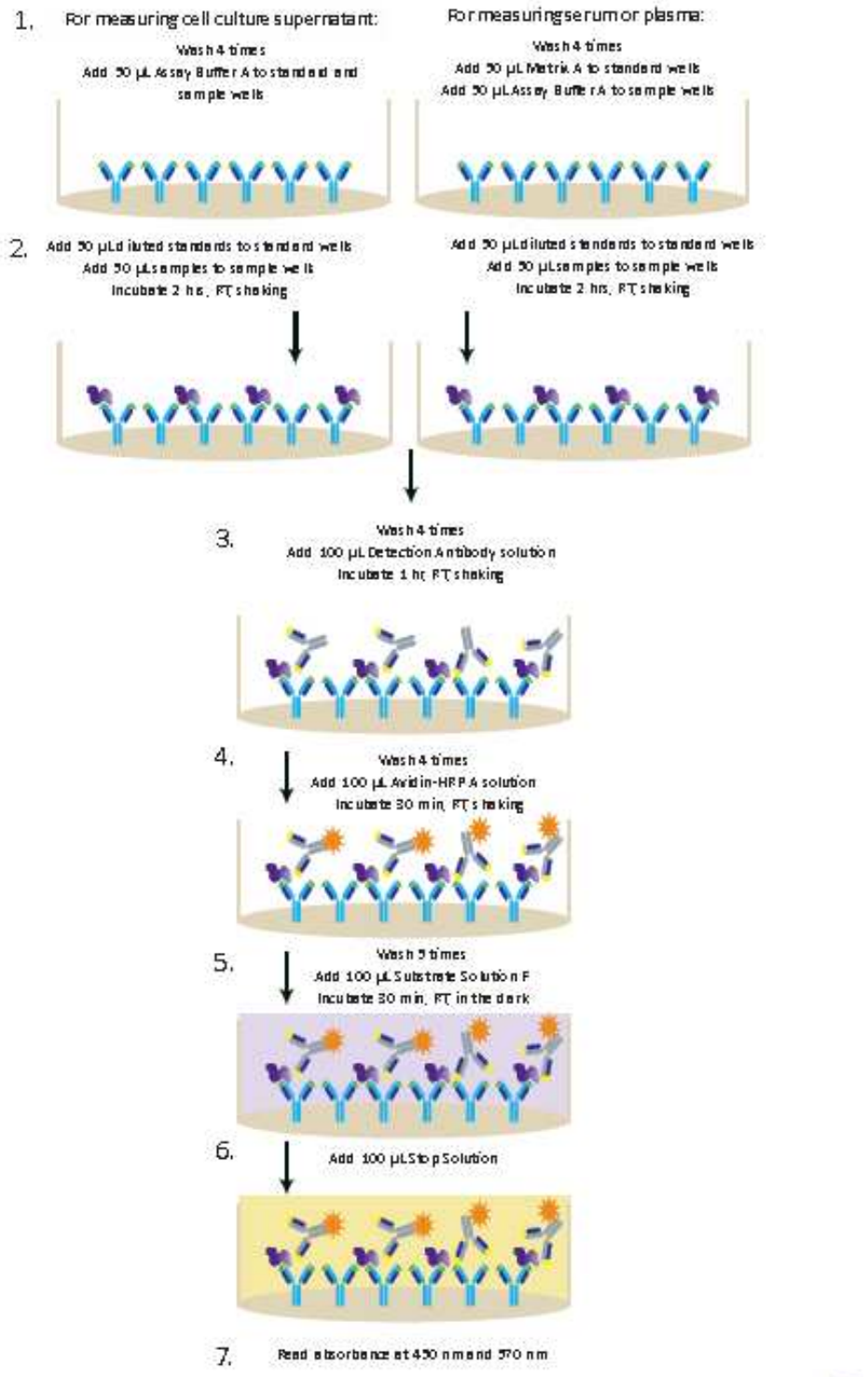
11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For these 5 final washes, soak wells in 1X Wash Buffer for 30 seconds to 1 minute during each wash. This will help minimize background.

12. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 30 minutes in the dark. Wells containing human IL-12 (p70) should turn blue in color with intensity proportional to its concentration. It is not necessary to seal the plate during this step.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

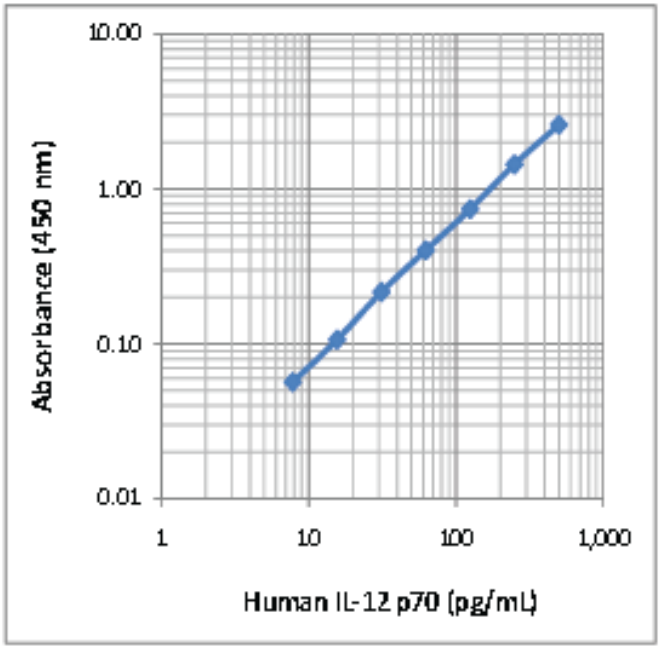
Assay Procedure Summary



Data Analysis

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.
If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:
This standard curve was generated for demonstration purposes only. A standard curve must be run with each assay.



Product Performance

Specificity: No cross reactivity was observed when this kit was used to analyze the following human and mouse recombinant cytokines/chemokines at up to 50 ng/mL.

Human	IL-1a IL-1b, IL-2, IL-6, IL-8, IL-10, IL-12 (p40), IL-13, IL-15, IL- 17A, IL-17E, IL-22, IL-23, IL-32a, FGF-basic, IFN-g, TNF-a, TSLP
Mouse	IL-12 (p40), IL-12 (p70)

Sensitivity: The average minimum detectable concentration of IL-12 (p70) plus two standard deviations is 1.2 pg/mL.

Recovery: Recombinant IL-12 (p70) (500, 125, and 31.3 pg/mL) was spiked into 10 human serum samples and analyzed with the Human IL-12 (p70) ELISA kit. On average, 100.3 % of the cytokine was recovered from the serum samples.

Linearity: Six serum samples with high concentrations of IL-12 (p70) were diluted with Matrix A to produce sample concentrations within the dynamic range of the assay. On average, 109.6% of the expected cytokine was detected from diluted serum samples.

Intra-Assay Precision: Sixteen replicates each of two samples containing different IL-12 (p70) concentrations were tested in one assay

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	244	61.75
Standard Deviation	8.16	3.69
% CV	3.34	5.96

Inter-Assay Precision: Two samples containing different concentrations of IL-12 (p70) were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	251.5	64.5
Standard Deviation	10.5	6.35
% CV	4.17	9.85

Biological Samples:

- *Serum/Plasma* - Normal human serum and plasma samples (n = 33) were assayed for basal levels of human IL-12 (p70). All samples measured less than the lowest IL-12 (p70) standard curve point, 7.8 pg/mL.
- *Cell Culture Supernates* - Human peripheral blood mononuclear cells at a concentration of $2x10^6$ cells/mL were co-stimulated with 1 ug/mL of LPS and 2.5 ug/mL R848 at 37℃ for 3 days. The cell culture supernatants were collected and assayed for the concentration of natural human IL-12 (p70). The concentration of human IL-12 (p70) was 360 pg/mL in LPS-R848 stimulated samples and undetectable in unstimulated samples.

Trouble Shooting Guide:

Problem	Probable Cause	Solutions
High Background	Background wells may have been contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and
	Washes may be insufficient	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	Substrate Solution F may have been contaminated	Substrate Solution F should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution may have been left out	Rerun the assay and follow the protocol.
	Wrong reagent or reagents may have been added in wrong sequential order	
	Plate agitation may not be sufficient	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer may contain Sodium Azide (NaN_3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations may have been performed at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard may have been incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard may have been inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70℃. Avoid repeated freeze-thaw cycles.
	Reagents may have been added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Trouble Shooting Guide:

Problem	Probable Cause	Solutions
Signal is high, standard curves have saturated signal	Standard may have been reconstituted with less volume than required	Correct volume of solution to that recommended in the protocol by adding additional Buffer A.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution may have been incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples may contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples may contain analyte concentrations greater than highest standard point	Samples require dilution before analysis.
High variation in samples and/or standards	Multichannel pipette may not be accurate	Confirm that pipette calibrations are accurate.
	Plate washing may not be adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Samples may not be homogenous	Thoroughly mix samples before assaying.
	Samples may contain high amount of particulate matter	Remove particulate matter by centrifugation.
	Possible cross-well contamination	Do not reuse plate sealers. Always change tips before performing a new reagent addition. Ensure that pipette tips do not touch contents in the wells during reagent additions.

Microtiter Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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