IL-12p70 (human), ELISA Kit

Catalog # ADI-900-202

96 Well Enzyme-linked Immunosorbent Assay Kit
For use with serum, plasma, and culture supernatants

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

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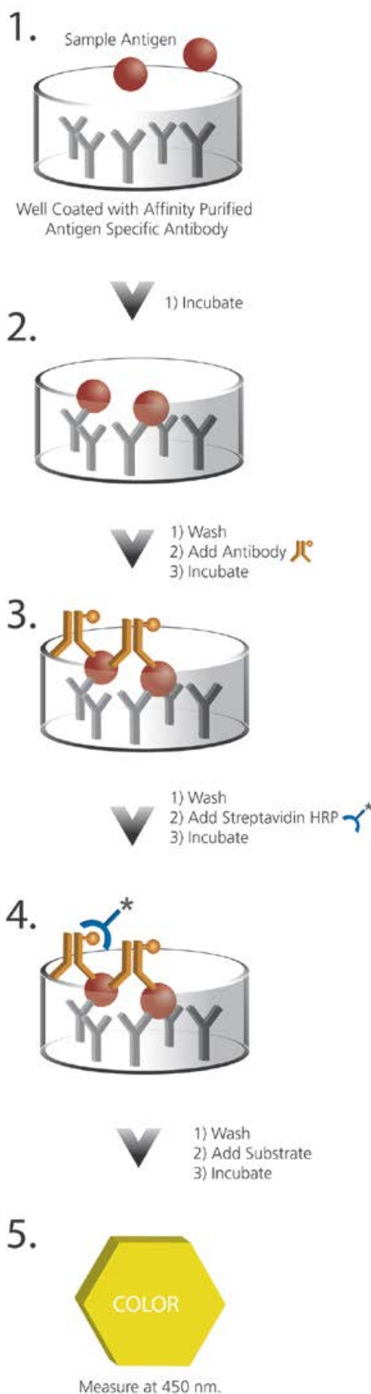


Introduction

The Enzo Life Sciences IL-12 Enzyme-linked Immunosorbent Assay (ELISA) kit is a complete kit for the quantitative determination of IL-12 in plasma, serum, and cell supernatants. Please read the complete kit insert before performing this assay.

Interleukin-12 (IL-12) belongs to the IL-12 cytokine family, which includes IL-12, IL-23, IL-27, and IL-35[1]. IL-12, a disulfide-linked 70 kDa protein composed of the subunits p40 and p30, is primarily produced by antigen-presenting cells (APCs), such as macrophages, monocytes, and dendritic cells (DCs), following their activation by recognition of pathogen-specific patterns[2]. IL-12 induces secretion of IFN-γ and promotes T-helper 1 (Th1) cell proliferation and differentiation[2].

Principle

1. Samples and standards are added to wells coated with a rat monoclonal antibody to IL-12p70. The plate is then incubated.
2. The plate is washed, leaving only bound IL-12 on the plate. A yellow solution of biotinylated mouse monoclonal antibody to IL-12p40 is then added. This binds to the IL-12 captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess biotinylated antibody. A blue solution of streptavidin conjugated to horseradish peroxidase is added to each well, binding to the biotinylated monoclonal IL-12p40 antibody.
4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of IL-12p70 in the sample.

Materials Supplied

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

1. **Assay Buffer 28**
   50 ml, Catalog No. 80-1599
   Phosphate buffered saline containing protein and detergents

2. **IL-12 (human), Standard**
   2 x 0.5 ml, Catalog No. 80-2358
   2 vials, each containing a 500 pg/ml solution of recombinant human IL-12

3. **IL-12p70 Clear Microtiter Plate**
   One plate of 96 wells, Catalog No. 80-2356
   A clear plate of break-apart strips coated with a monoclonal antibody specific for IL-12p70

4. **IL-12p40 ELISA Antibody**
   10 ml, Catalog No. 80-2359
   A yellow solution of biotinylated monoclonal antibody to IL-12p40

Activity of conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

5. **IL-12p70 ELISA Conjugate**
   10 ml, Catalog No. 80-2360
   A blue solution of streptavidin conjugated to horseradish peroxidase

6. **Wash Buffer Concentrate**
   100ml, Catalog No. 80-1287
   Tris buffered saline containing detergents

Protect substrate from prolonged exposure to light.

7. **TMB Substrate**
   10 ml, Catalog No. 80-0350
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide

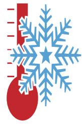
Stop solution is caustic. Keep tightly capped.

8. **Stop Solution 2**
   10 ml, Catalog No. 80-0377
   A 1N solution of hydrochloric acid in water

9. **IL-12 Assay Layout Sheet**
   1 each, Catalog No. 30-0297

10. **Plate Sealer**
    3 each, Catalog No. 30-0012

Storage



Reagents require separate storage conditions.

All components of this kit, **except the Standard**, are stable at 4°C until the kit's expiration date. The standard must be stored at or below -20°C upon receipt. Shipping conditions may not reflect recommended storage conditions.

Materials Needed but Not Supplied

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable polypropylene beakers
5. Graduated cylinders
6. A microplate shaker
7. Lint-free paper for blotting
8. Microplate reader capable of reading 450 nm
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit, such as Assay Blaster! assay analysis software (Cat. #ADI-28-0002).

Reagent Preparation

Bring all reagents to room tempera-ture for at least 30 minutes prior to opening.

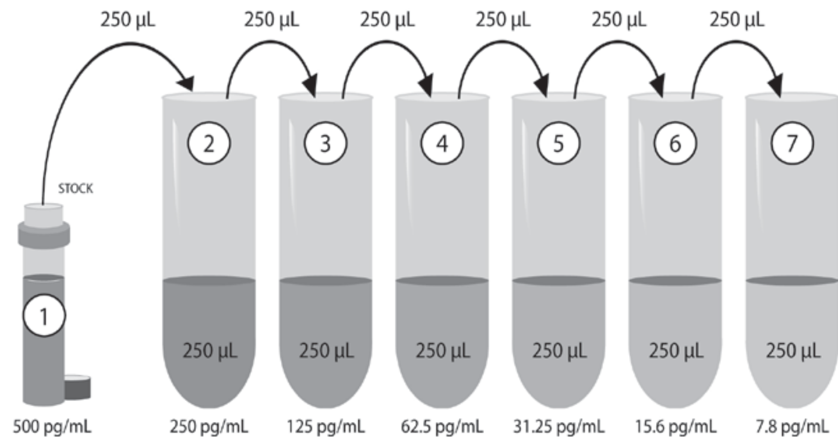
If buffers other than those provided are used in the assay, the end-user must determine the ap-propriate dilution and assay valida-tion.

1. **Wash Buffer**
   Prepare the wash buffer by diluting 50 ml of the supplied Wash Buffer Concentrate with 950 ml of deionized water. This can be stored at room temperature until the kit's expiration date, or for 3 months, whichever comes first.
2. **Human IL-12 Standards**
   Allow a 500µl vial of human IL-12 standard to thaw. Spin briefly to transfer entire volume to base of vial before use.

Plastic tubes must be used for standard preparation.

Label six disposable polypropylene 12 x 75 mm tubes #2 through #7. Pipet 250 µl of Assay Buffer 28 into each tube. Remove 250 µl from stock vial and add to tube #2 and vortex thoroughly. Remove 250 µl from tube #2 and add to tube #3. Vortex thoroughly. Continue this for tubes #3 through #7.

The concentration of human IL-12 in tubes #1 through #7 will be 500, 250, 125, 62.5, 31.25, 15.6 and 7.8 pg/ml respectively (see image below).



Diluted standards should be used within 1 hour of preparation. The concentrations of the standards are labeled above.

Sample Handling

The IL-12 (human), ELISA kit is compatible with IL-12 samples in culture supernatants, plasma, and serum of human origin. Samples diluted sufficiently into the assay buffer can be read directly from a standard curve. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. A minimum 1:8 dilution is recommended for plasma, 1:4 for serum while cell culture supernates (with 10% serum supplement) may be run diluted 1:2. These are the minimum recommended dilutions to remove matrix interference in the assay. Samples may require a

greater dilution to remove interference or to be read within the dynamic range. The optimal dilution for any experiment should be determined by the investigator.

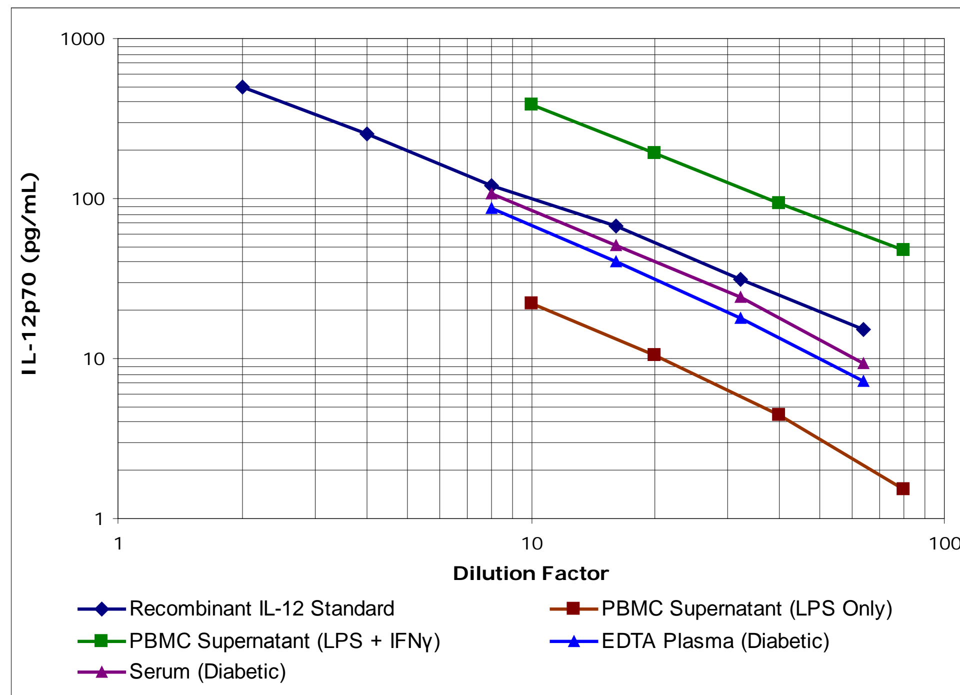
Dilutional Linearity

The minimum recommended dilution for several common samples was determined by serially diluting samples spiked with recombinant human IL-12p70 into the assay buffer and identifying the dilution at which linearity was observed. The assay buffer was spiked to the same concentration and used as a control to determine linearity.

	Average % of Expected		
Dilution Factor	**Culture media + 10% FBS**	**EDTA Plasma (Pooled)**	**Serum (Pooled)**
Neat	74.4	---	---
1:2	91.5	---	---
1:4	97.5	72.5	88.4
1:8	99.0	88.5	96.1
1:16	102.6	98.4	102.3
1:32	110.9	104.0	108.8
1:64	74.6	105.1	113.7
1:128	---	109.5	131.4

Parallelism

Parallelism experiments were carried out to determine if the recombinant human IL-12 standard accurately determines IL-12 concentrations in biological matrices. Stimulated peripheral blood mononuclear cell (PBMC) culture supernatants as well as paired diabetic serum and plasma samples were used to assess parallelism. Samples were serially diluted in the assay buffer and IL-12p70 levels were assessed from a standard curve using four parameter logistic curve fitting. The observed values were plotted against the dilution factors. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples.



Spike and Recovery

After diluting each sample matrix to its minimum recommended dilution, recombinant human IL-12 was spiked at high, medium, and low concentrations. The recovery of the standard in spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean and range of percent recovery at the three concentrations are indicated below for each matrix.

Sample Matrix (# of samples)	Minimum Required Dilution	Spike Concentration (pg/ml)	Recovery of Spike (Range)
Serum (n =5)	1:4	175	96.6% (83-108%)
		87.5	96% (82-110%)
		43.75	93% (76-106%)
Human EDTA Plasma (n=5)	1:8	175	105% (90-112%)
		87.5	100% (86-106%)
		43.75	100% (80-133%)
TCM + 10% FBS (n=1)	1:2	175	106% (n/a)
		87.5	104% (n/a)
		43.75	100% (n/a)

Plasma Preparation

Samples must be stored at or below -20˚C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

1. Collect whole blood in appropriate tube.
2. Centrifuge at 1000 x g for 15 minutes at 4˚C.
3. Place supernatant in a clean tube.
4. The supernatant may be aliquoted and stored at or below -20˚C, or used immediately in the assay.

PBMC (Peripheral Blood Mononuclear Cells) Supernate Preparation

1. Incubate PBMCs in RPMI supplemented with 10% FBS, L-glutamine and pen/strep/ampho for 24 hours.
2. Replace media with media containing IFN-γ at 100ng/ml. Incubate for 16 hours.
3. Add LPS at 1µg/ml. Incubate for 24 hours.
4. Collect cell supernatant.
5. Centrifuge at 1700 rpm for 10 minutes at room temperature to pellet any cells or cellular debris.
6. Collect supernatant in a clean tube.
7. The supernatant may be aliquoted and stored at or below -20˚C, or used immediately in the assay.

Assay Procedure

Bring all reagents to room tempera-ture for at least 30 minutes prior to opening.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and rea-gent.

All standards and samples should be run in duplicate.

Pipet the reagents to the sides of the wells to avoid pos-sible contamina-tion.

Prior to the addi-tion of antibody, conjugate, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in

Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the foil pouch and seal the ziploc. Store unused wells at 4°C.

1. Pipet 100 µl of the assay buffer into the S0 (0 pg/ml standard) wells.
2. Pipet 100 µl of Standards #1 through #7 into the appropriate wells.
3. Pipet 100 µl of the Samples into the appropriate wells.
4. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
5. Empty the contents of the wells and wash by adding 400 µl of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100 µl of yellow biotinylated antibody into each well, except the Blank.
7. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
8. Wash as above (Step 5).
9. Add 100 µl of blue conjugate to each well, except the Blank.
10. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
11. Wash as above (Step 5). Pipet 100 µl of the substrate solution into each well.
12. Seal the plate. Incubate at room temperature on a plate

shaker for 30 minutes at ~500 rpm.

13. Pipet 100 µl the stop solution to each well.

14. Zero the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Calculation of Results

Several options are available for the calculation of the concentration of IL-12p70 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. Assay Blaster! assay analysis software (Cat. #ADI-28-002) is an easy-to-use and cost effective program that provides the options of point-to-point, 4PL and 5PL curve fitting options.

The concentration of IL-12p70 can be calculated as follows.

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

   $$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Plot the Percent Bound (B/Bo) versus concentration of IL-12p70 for the standards. Approximate a straight line through the points. The concentration of IL-12p70 of the unknowns can be determined by interpolation.

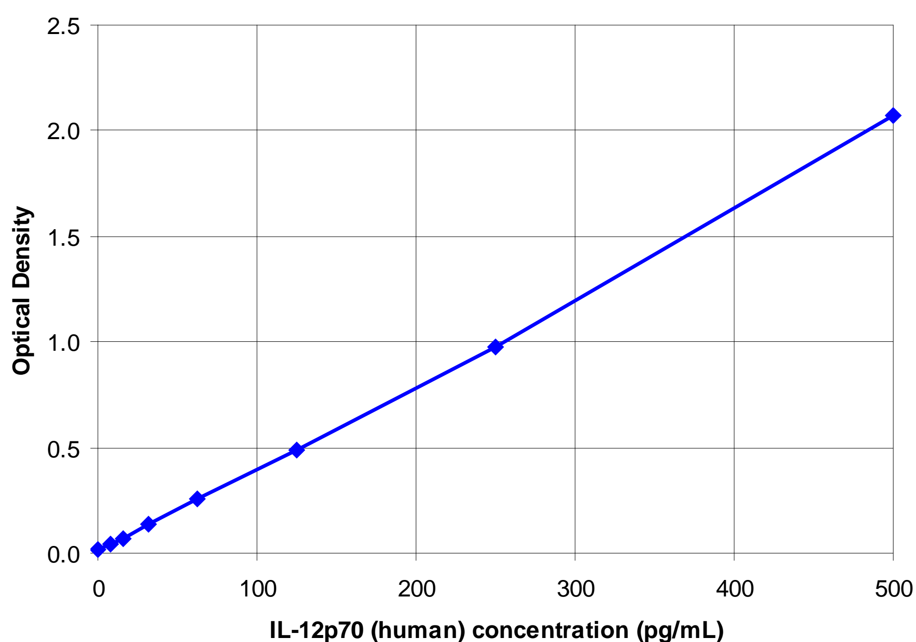
Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	IL-12(pg/ml)

S0	0.018	0
S1	2.069	500
S2	0.965	250
S3	0.482	125
S4	0.254	62.5
S5	0.137	31.25
S6	0.072	15.6
S7	0.045	7.8
Unknown 1	0.720	185.0
Unknown 2	0.200	51.9



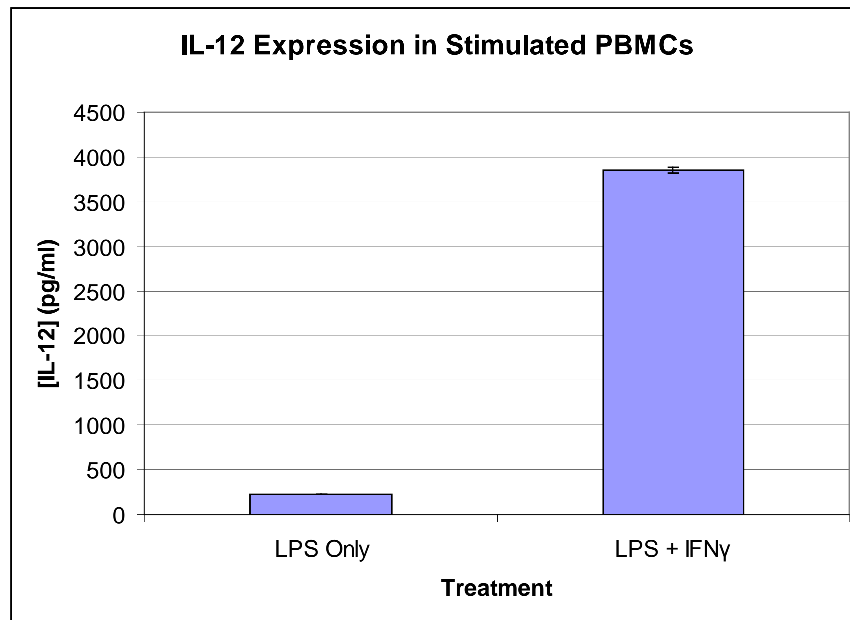
Performance Characteristics

Specificity

The cross reactivities for related compounds were determined by diluting cross reactants in the assay buffer at several concentrations. These cross reactants were then measured in the assay.

Cross Reactant	% Cross-reactivity
IL-23 (human)	≤0.1
IL-12 (mouse)	≤0.0156
IL-10 (human)	≤0.0331
IL-17 (human)	≤0.0331
IL-13 (human)	≤0.011
IL-21 (human)	≤0.011
Il-12p40 (human)	≤0.01
IL-2 (human)	≤0.0037
IL-15 (human)	≤0.0037
IL-29 (human)	≤0.0037

Stimulation Experiment



PBMCs were stimulated with IFN-γ and LPS as described above. Supernatants were diluted to the MRD in assay buffer and the levels of IL-12p70 were determined in the assay. IL-12p70 was undetectable in unstimulated control samples.

Sensitivity

The sensitivity or limit of detection of the assay is 0.90 pg/ml. The sensitivity was determined by interpolation at two standard deviations above the mean signal at background (0 pg/ml) using data from 6 standard curves.

Precision

Intra-assay precision was determined in by assaying 16replicates of three buffer controls containing IL-12 in a single assay.

pg/ml	%CV
180.9	4.3
91.7	3.3
47.2	4.6

Inter-assay precision was determined by measuring buffer controls of varying IL-12 concentrations in multiple assays over several days.

pg/ml	%CV
185.0	1.5
97.6	5.7
51.9	10.1

References

1. Interleukin-35: odd one out or part of the family?: L.W. Collison & D.A. Vignali; Immunol. Rev. **226**, 248 (2008).
2. Interleukin-12 family members and the balance between rejection and tolerance: S. Goriely & M. Goldman; Curr. Opin. Organ Transplant. **13**, 4 (2008).
3. Regulation of Interleukin-12 Expression in Human Monocytes: Selective Priming by Interferon-γ of Lipopolysaccharide-Inducible p35 and p40 Genes: M. Hayes, J. Wang, M.A. Norcross. Blood, **86**, 2 (1995)

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TRADEMARKS AND PATENTS

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