ELISA PRODUCT INFORMATION & MANUAL

Human IL-12 ELISA Kit (Colorimetric)

NBP3-20185

Sample insert for reference use only

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

BACKGROUND 1
INTENDED USE 1
PRINCIPLE OF THE ASSAY 2
MATERIALS PROVIDED 3
STORAGE 4
OTHER SUPPLIES REQUIRED 5
PRECAUTIONS 6
SAFETY INSTRUCTIONS 6
TECHINICAL TIPS 6
TYPICAL DATA 11
PRECISION 12
RECOVERY 12
LINEARITY 12
SENSITIVITY 13
CALIBRATION 13
SAMPLE VALUES 14
SPECIFICITY 14
TROUBLE SHOOTING 15

BACKGROUND

Interleukin-12 (IL-12), also known as natural killer cell stimulatory factor (NKSF) or cytotoxic lymphocyte maturation factor (CLMF), is a 70 kDa disulfide-linked heterodimeric cytokine composed of a 35-kD subunit P35 and a 40-kD subunit P40, also designated as IL-12A (Interleukin-12 subunit alpha) and IL-12B (Interleukin-12 subunit beta). IL-12 is predominantly produced by macrophages and B lymphocytes and plays an important role in the activities of natural killer cells and T lymphocytes. IL-12 is involved in the differentiation and development of Th1 cells, enhancement of natural killer cytolytic function and mitogenic effects, as well as induction of IFN-gamma during which it can synergize with other IFN-gamma inducers. The most powerful inducers of IL-12 production are bacteria, bacterial products and parasites. IL-12A shows significant sequence similarity to IL-6, G-CSF, and exerts biological activities only when the IL-12B is co-expressed. IL-12B deficient mice are resistant to the induction of experimental chronic inflammatory diseases whereas IL-12A knock-out mice develop more severe forms, suggesting opposite functions of the two subunits in the outcome of chronic inflammatory diseases.

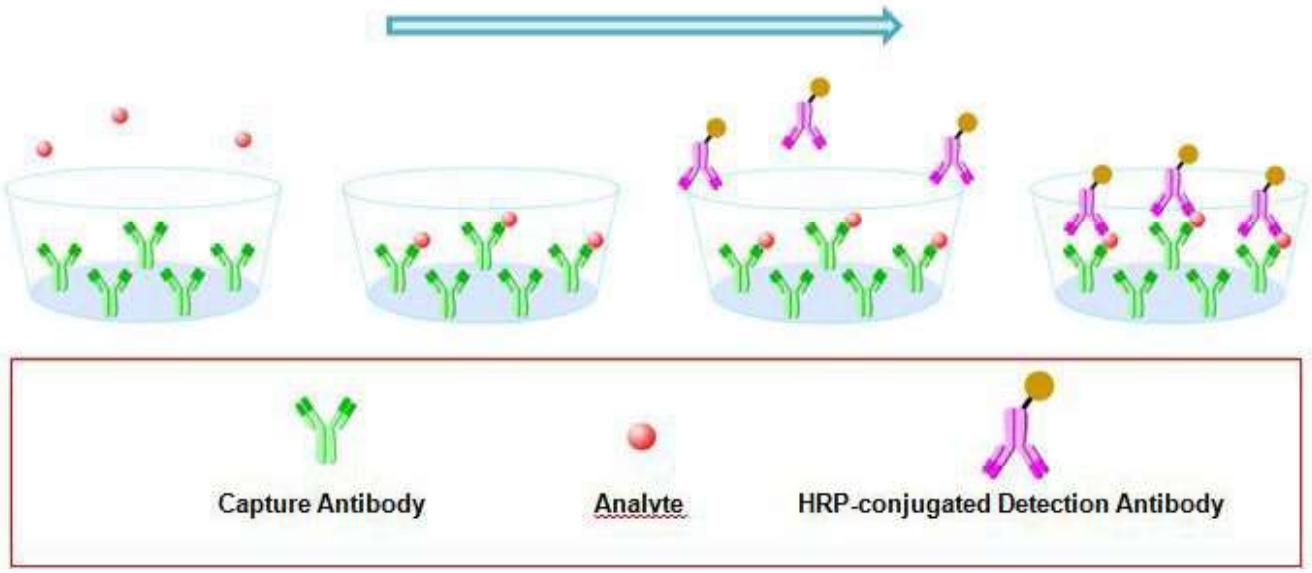
INTENDED USE

For the quantitative determination of Human IL-12 concentration in cell culture supernates.

The use of this kit for other sample types need be validated by the end user due to the complexity of natural targets and unpredictable interference.

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for Human IL-12 (IL-12A & IL-12B Heterodimer) has been pre-coated onto well plate strips. Standards and samples are added to the wells and Human IL-12 present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Human IL-12 antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Human IL-12 bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).



MATERIALS PROVIDED

Human IL-12 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with rabbit mAb antibody against Human IL-12.

Human IL-12 Detection Antibody - 0.2 mg/mL of rabbit mAb antibody against Human IL-12 conjugated to horseradish peroxidase (HRP) with preservatives.

Human IL-12 Standard - Recombinant Human IL-12 in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.

Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.

Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.

1×Standard Dilution Buffer - 2 mL

Color Reagent A - 13 mL of stabilized hydrogen peroxide.

Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution - 12 mL of 2 N sulfuric acid.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8℃ and the kit is stable for 6 months upon receipt.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="3">Stored for up to 1 week at 2 - 8℃</td></tr>
<tr><td>Diluted Dilution Buffer</td></tr>
<tr><td>1×Standard Dilution Buffer</td></tr>
<tr><td>Conjugate</td><td rowspan="4">Stored for up to 1 month at 2 - 8℃</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td><td>After reconstitution, store for up to 1 month at -80℃.
The reconstituted standards should be aliquoted and
avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.
Stored for up to 1 month at 2 - 8℃</td></tr>
</table>

OTHER SUPPLIES REQUIRED

·Microplate reader capable of measuring absorbance at 450 nm

·Pipettes and pipette tips

·Deionized or distilled water

·Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer

·500 mL graduated cylinder

·Tubes for standard dilution

·Well plate cover or seals

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.

2. The kit should not be used beyond the expiration date.

3. Do not mix reagents from different lots.

4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

7. Personal protective equipment such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

8. Bring all reagents and samples to room temperature before use.

9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.

10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.

11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.

12. Read the absorbance of each well within 20 minutes after adding the stop solution.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze-thaw cycles. If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.**

Note:

The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Detection Antibody - Centrifuge at 10,000 x g for 20 seconds. Dilute to **work concentration** of 0.3 μg/mL in Dilution Buffer before use.

Substrate Solution **-** Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 μL of the resultant mixture is required per well. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**

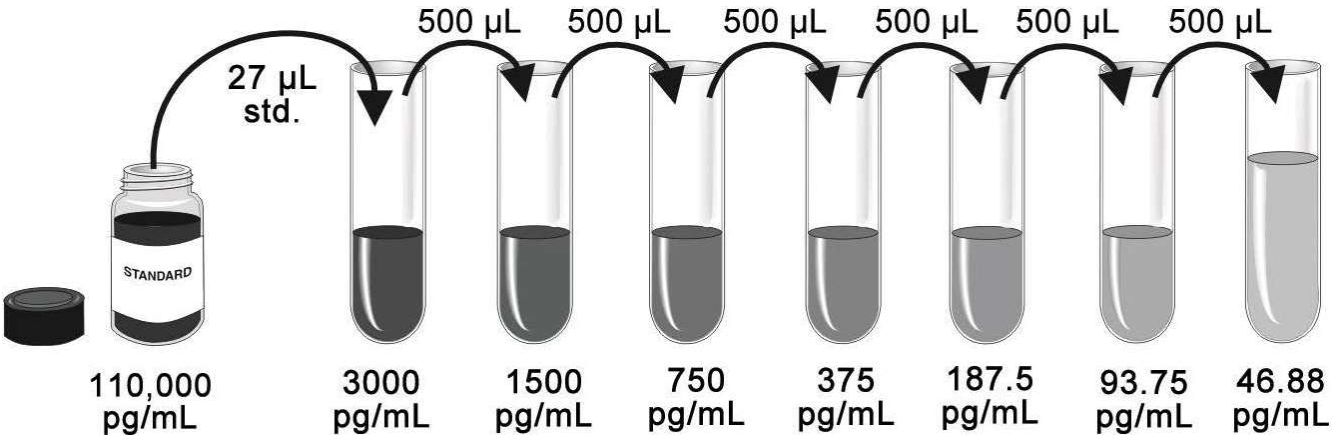
Human IL-12 Standard - Reconstitute the Human IL-12 Standard with 1 mL standard dilution buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (**Do not turn the vial upside down**). Mix the standard to ensure

complete reconstitution prior to making dilutions.

Prepare serially diluted standards as described in the following step:

Pipette 953 µL of Dilution Buffer into the 3000 pg/mL tube. Pipette 500 µL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 3000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). **Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.**

The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 µL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.**

4. Add 100 µL of each serially diluted protein standard or test sample per well including a zero standard. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

8. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature**. Protect from light.**

9. Add 100 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

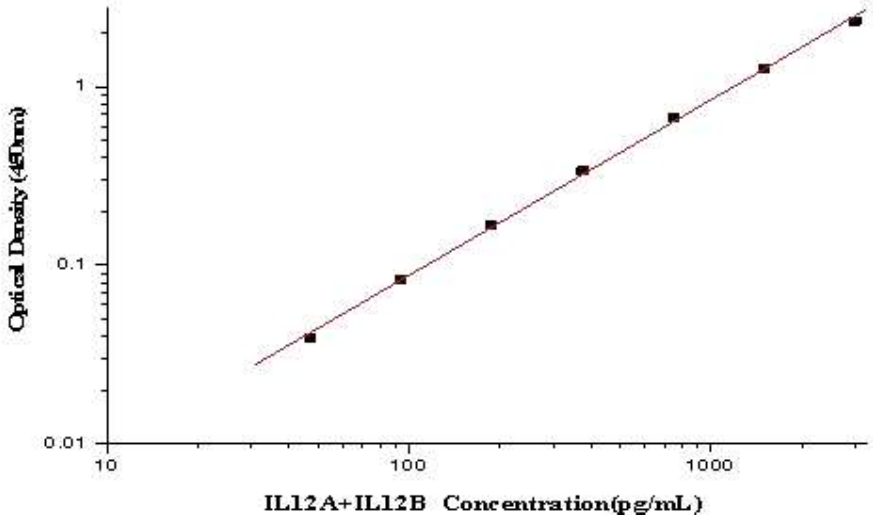
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

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. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
46.88	0.039
93.75	0.083
187.5	0.169
375	0.338
750	0.666
1500	1.259
3000	2.324



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 five separate assays to assess inter-assay precision.

	Intra -assay Precision			**Inter -assay Precision**		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	405	765	1473	371	705	1568
SD	15.44	43.03	108.80	8.49	41.51	69.30
CV (%)	3.8%	5.6%	7.4%	2.3%	5.9%	4.4%

RECOVERY

The recovery of Human IL-12 spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	**Average % Recovery**	**Range**
cell culture supernates (n=3)	103	98 -106%

LINEARITY

		Cell culture supernates
1:2	recovery of detected	91%
1:4	recovery of detected	96%
1:8	recovery of detected	103%
1:16	recovery of detected	104%

SENSITIVITY

The minimum detectable dose (MDD) of Human IL-12 is typically less than 7.55 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified HEK 293-expressed recombinant Human IL-12.

SAMPLE VALUES

Human peripheral blood mononuclear cells （2 x 10E6 cells/mL） were cultured in DMEM supplemented with 5% bovine calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate, stimulated with 10 μg/mL PHA for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of natural IL-12, and measured 0.34 ng/mL.

SPECIFICITY

This assay recognizes recombinant Human IL-12A-his+IL-12B. The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed.

Recombinant human			
IL-1	IL-2	IL-33	IL-10
IL-8	TNF-α	IL-4	IL-6
IFN-γ	MIP-1β	TIMP-1	TIMP-2
GM-CSF	SCF	PDGF	VEGF
GCSFb	IL27	IL6R	IL12B
IL12RB1			
Recombinant mouse			
IL6	IL27	IL12B	IL12Bh(m)+IL23Ah
IL12A+IL12B			

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	Color Reagent should be clear and colorless prior to addition to wells	Color Reagent should be clear and colorless prior to addition to wells
	Use clean tubes and pipettes tips	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples