IL-12p70 High Sensitivity ELISA Kit (Human) (OKDB00043)

Instruction for Use

For the quantitative measurement of IL-12p70 in serum, plasma, buffered solutions or cell culture medium.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology IL-12p70 High Sensitivity ELISA Kit (Human) (OKDB00043) is designed for the highly sensitive in-vitro quantitative measurement of recombinant or natural IL-12p70 in human serum, plasma buffered solutions or cell culture medium. The kit is based on standard sandwich enzyme-linked immuno-sorbent assay methods along with an additional detection process employing a polymerization step to amplify the number of detector bound HRP molecules. A monoclonal antibody specific for IL-12p70 has been pre-coated into wells of a 96-well plate. Standards or test samples are added to the wells and incubated. After washing, a biotinylated detector antibody specific for IL-12p70 is added, incubated and followed by washing. Streptavidin-HRP conjugate is then added, incubated and unbound conjugate is washed away. Amplification is achieved by adding a Biotin-Tyramine reagent, incubating and washing, followed by a second incubation with Streptavidin-HRP conjugate. An enzymatic reaction is then produced through the addition of TMB substrate which is catalyzed by HRP to generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample IL-12p70 captured in well.

Target Background

The protein encoded by this gene is a secreted cytokine that is important for the proliferation of T and B lymphocytes. The receptor of this cytokine is a heterotrimeric protein complex whose gamma chain is also shared by interleukin 4 (IL4) and interleukin 7 (IL7). The expression of this gene in mature thymocytes is monoallelic, which represents an unusual regulatory mode for controlling the precise expression of a single gene. The targeted disruption of a similar gene in mice leads to ulcerative colitis-like disease, which suggests an essential role of this gene in the immune response to antigenic stimuli.

General Specifications

General Specifications	
Range	0.78 – 25 pg/mL
LOD	< 0.75 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Human IL-12p70 UniProt ID: P29459 GeneID: 3592 Target Alias: P35, CLMF, NFSK, NKSF1, IL-12A

2. Storage and Stability

• Upon receipt store kit at 4°C until expiration date on packaging.

3. Kit Components

• The following reagents are the provided contents of the kit.

Reagents	Quantity			Handling Requirements
	1 x 48 Well Kit	1 x 96 Well Kit	2 x 96 Well Kit	
IL-12p70 Well Strip Microplate	6 x Wells	12 x 8 Wells	24 x 8 Wells	Ready to use (Pre-coated)
IL-12p70 Standard	1 Vial	2 Vials	4 Vials	Reconstitute as directed in Step 9.4
10X Standard Diluent (Buffer)	1 x 25 mL	1 x 25 mL	1 x 25 mL	Dilute as directed in Step 9.2
Standard Diluent (Serum)	1 x 7 mL	1 x 7 mL	2 x 7 mL	Ready to use
27.5X Biotinylated anti-IL-12p70 Detector Antibody	1 x 400 µL	1 x 400 µL	2 x 400 µL	Dilute as directed in Step 9.3
Biotinylated Detector Antibody Diluent	1 x 7 mL	1 x 7 mL	1 x 13 mL	Ready to use
Streptavidin-HRP	1 x 5 µL	2 x 5 µL	4 x 5 µL	Prepare as directed in Step 9.5
Amplification Diluent	1 x 25 mL	1 x 25 mL	1 x 25 mL	Ready to use
100X Amplifier	1 x 200 µL	1 x 200 µL	2 x 200 µL	Dilute as directed in Step 9.6
HRP Diluent	1 x 25 mL	1 x 25 mL	2 x 25 mL	Ready to use
200X Wash Buffer	1 x 10 mL	1 x 10 mL	2 x 10 mL	Dilute as directed in Step 9.1
TMB Substrate	1 x 11 mL	1 x 11 mL	1 x 24 mL	Ready to use
H_2SO_4 Stop Reagent	1 x 11 mL	1 x 11 mL	2 x 11 mL	Ready to use

4. Precautions

• Read instructions fully prior to beginning use of the assay kit.
• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
• Kit cannot be used beyond the expiration date on the label.

5. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.
• 37°C Incubator (optional)

6. Technical Application Tips

• Do not mix or substitute components from other kits.
• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
• Replicate wells are recommended for standards and samples.
• Cover microplate while incubating to prevent evaporation.
• Do not allow the microplate wells dry at any point during the assay procedure.
• Do not reuse tips or tube to prevent cross contamination.
• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
• Completely remove of all liquids when washing to prevent cross contamination.
• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
• Equilibrate all materials to ambient room temperature prior to use (standards exception).
• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
• Pipetting less than 1 µL is not recommended for optimal assay accuracy.
• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
• Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

7. Sample Preparation

7.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
• Samples not indicated in the manual must be tested to determine if the kit is valid.
• Prepare samples as follows:

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Cell culture supernatants and other biological fluids** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

7.2 Sample Dilution

• Depending on the type of samples you are assaying, the kit includes two standard diluents. Because biologicals fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure. You should reconstitute standard vials with the most appropriate standard diluent as follows:

Sample Type	Sample Diluent
Serum or Plasma	Standard Diluent (Serum)
Cell Culture Supernatants	1X Standard Diluent (Buffer)

• Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted. Note that a dilution of at least 1:2 will mitigate interference factors.
• Dilute samples using **1X Sample Diluent**.
• Mix diluted samples gently and thoroughly.
• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

8.1 1X Wash Buffer

8.1.1 Equilibrate **200X Wash Buffer** concentrate to room temperature and mix gently.

8.1.2 Add the entire 10 mL contents of the **200X Wash Buffer** bottle to 1990 mL of ultra-pure water to a clean > 2,000 mL bottle, graduated cylinder or other vessel.

8.1.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.1.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.2 1X Standard Diluent Buffer

8.2.1 Equilibrate **10X Standard Diluent Buffer** concentrate to room temperature and mix gently.

8.2.2 Add the entire 25 mL contents of the **10X Standard Diluent Buffer** bottle to 225 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

8.2.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.2.4 Store the **1X Standard Diluent Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Standard Diluent Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.3 1X Biotinylated anti-IL-12p70 Detector Antibody

8.3.1 Prepare the **1X Biotinylated anti-IL-12p70 Detector Antibody** immediately prior to use by diluting the **27.5X Biotinylated anti-IL-12p70 Detector Antibody** 1:30 with **Biotinylated Detector Diluent** in volumes according to the table below.

8.3.2 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

Number of wells required	Biotinylated IL-12p70 Detector Antibody (µL)	Biotinylated Antibody Diluent (µL)
16	40	1,060
32	80	2,120
48	120	3,180
96	240	6,360

8.4 Human IL-12p70 Assay Standards

8.4.1 Prepare the IL-12p70 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.4.2 Reconstitute one vial of the provided **IL-12p70 Standard** for each experiment according using the volume indicated on the vial label. The diluent used to reconstitute the vial should be the same as was used to dilute the samples in Step 8.2 (**1X Standard Diluent (Buffer)** or **Standard Diluent (Serum)**) This will produce a stock solution of 25 pg/mL **IL-12p70 Standard.** Prepare the standard as follows:

8.4.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.4.2.2 Add the indicated volume of **1X Sample Diluent** to the vial.

8.4.2.3 Seal the vial then mix gently and thoroughly.

8.4.2.4 Leave the vial at ambient temperature for 15 minutes.

8.4.3 Prepare a set of seven serially diluted standards as follows:

8.4.3.1 Label tubes with numbers 2 – 8.

8.4.3.2 Use the undiluted 25 pg/mL **IL-12p70 Standard** as the high standard point (Tube #1).

8.4.3.3 Add 300 µL of **Sample Diluent** to Tube #2 – 8.

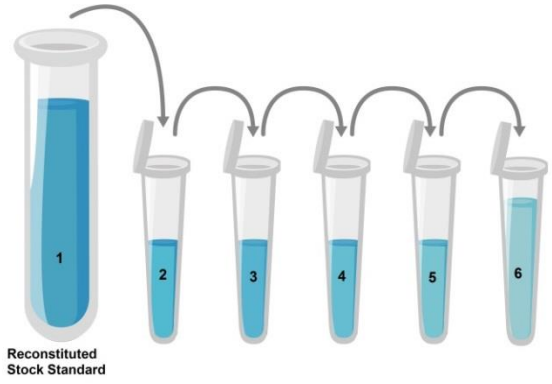
8.4.3.4 Prepare **Standard #2** by adding 300 µL of 25 pg/mL **IL-12p70 Standard** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.4.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.4.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.4.3.7 Tube #8 is a blank standard (only **Sample Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	25 pg/mL Reconstituted IL-12p70 Standard	NA	NA	NA	25 pg/mL
2	25 pg/mL	300	300	600	12.5 pg/mL
3	12.5 pg/mL	300	300	600	6.25 pg/mL
4	6.25 pg/mL	300	300	600	3.12 pg/mL
5	3.12 pg/mL	300	300	600	1.56 pg/mL
6	1.56 pg/mL	300	300	600	0.78 pg/mL
7	NA	0	300	600	NA



8.5 Streptavidin-HRP Solutions

8.5.1 Prepare the **Streptavidin-HRP Stock Solution** immediately prior to use by adding 500 µL of **HRP Diluent** . Mix gently and thoroughly. Do not retain this vial for future experiments.

8.5.2 The stock solution will be used to prepare **Streptavidin-HRP Solution** for the assay detection steps. Dilute the re-suspended **Streptavidin-HRP Stock Solution** as indicated on the table below for the appropriate number of wells being run in the experiment. Prepare this solution immediately prior to use.

8.5.3 Mix thoroughly and gently. Do not store for future use.

Number of experimental wells	**Streptavidin-HRP Solution** (For use in Step X)	
	Streptavidin-HRP Stock Solution (µL)	Streptavidin-HRP Diluent (mL)
16	13	1.95
24	25	3.75
32	26	3.9
48	50	7.5
96	80	12

8.6 1X Amplifier

8.6.1 Dilute the **100X Amplifier** into **Amplifier** Diluent according to the following table.

8.6.2 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.6.3 Use immediately prior to use and do not store.

Number of experimental wells	Amplifier (µL)	Amplier Diluent (mL)
16	20	1.98
32	40	3.96
48	60	5.94
96	120	11.88

8.7 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at °4C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

9.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

9.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **IL-12p70 Microplate**. At least two replicates of each standard, sample or blank is recommended.

9.3 Cover the plate with the well plate lid and incubate for 60 minutes with slow shaking at room temperature.

9.4 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.5 Wash plate 3 times with **1X Wash Buffer** as follows:

9.5.1 Add 300 µL of **1X Wash Buffer** to each assay well.

9.5.2 Incubate for 1 minute.

9.5.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

9.5.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.5.5 Repeat steps 10.5.1 through 10.4.4 **two** more times.

9.6 Add 50 µL of prepared **1X Biotinylated anti-IL-12p70 Detector Antibody** to each well.

9.7 Cover with the well-plate lid and incubate for 60 minutes with slow shaking at room temperature.

9.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.10 Wash plate as in Step 10.5.

9.11 Add 100 µL of prepared **Streptavidin-HRP Solution #1** into each well.

9.12 Cover with the well-plate lid and incubate for 20 minutes with slow shaking at room temperature.

9.13 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.14 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.15 Wash the plate as in Step 10.5

9.16 Add 100 µL of **1X Amplifier** to all wells.

9.17 Cover with the well-plate lid and incubate for 15 minutes with slow shaking at room temperature.

9.18 Wash the plate as in Step 10.5

9.19 Add 100 µL of **Streptavidin-HRP Solution #2** to all wells.

9.20 Cover with the well-plate lid and incubate for 20 minutes with slow shaking at room temperature

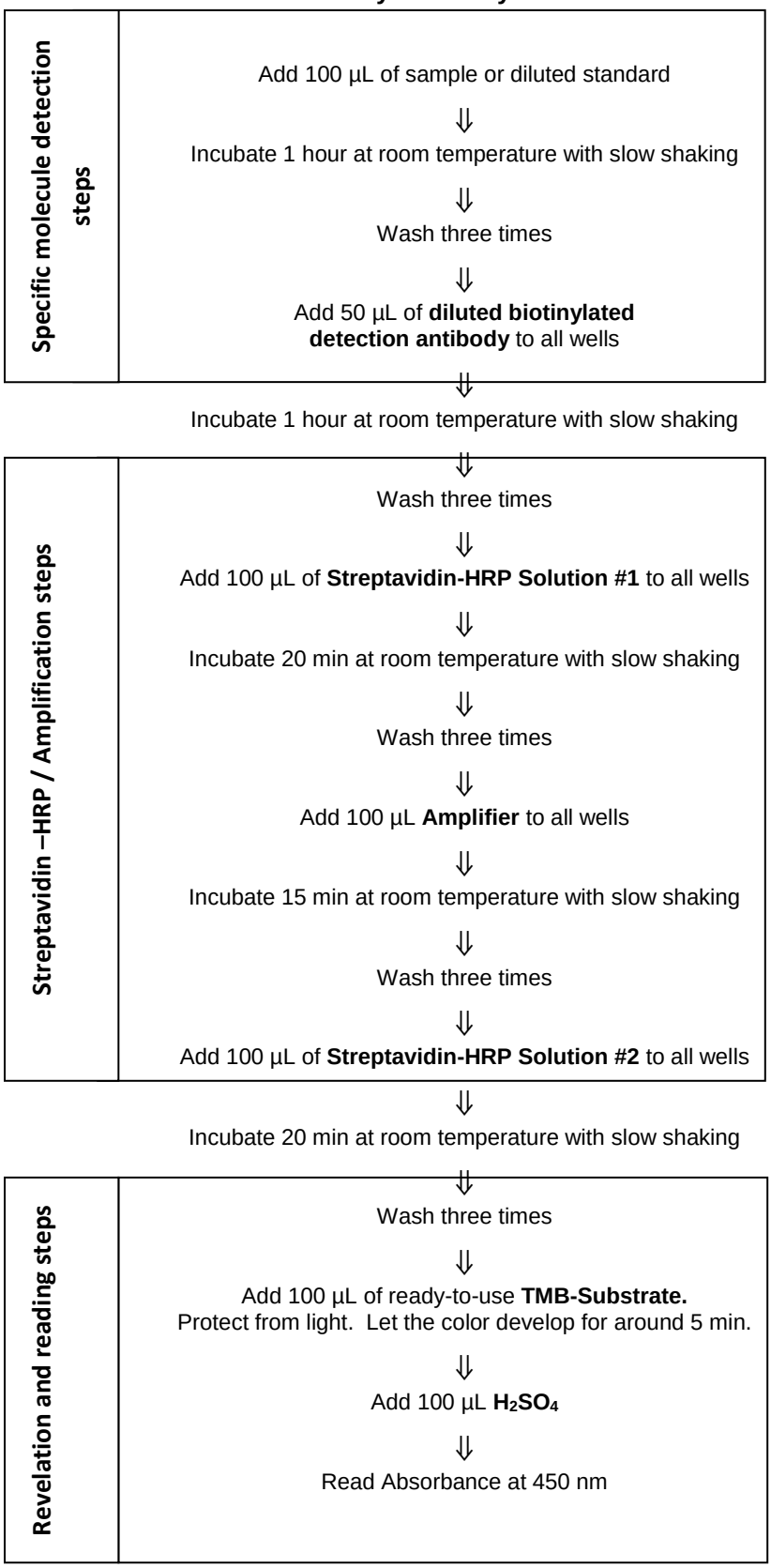
9.21 Add 100 µL of the **TMB Substrate Solution** to all wells.

9.22 Incubate in the dare for 10 – 20 minutes. Avoid direct light exposure by covering plate with aluminum foil. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.
(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

9.23 Add 100 µL of **H_2SO_4 Stop Reagent** into all wells. Well color should change to yellow immediately. Add the **H_2SO_4 Stop** in the same well order as done for the **TMB Substrate Solution**.

9.24 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 540 nm or 570 nm.

Assay Summary



10. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The IL-12p70 concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

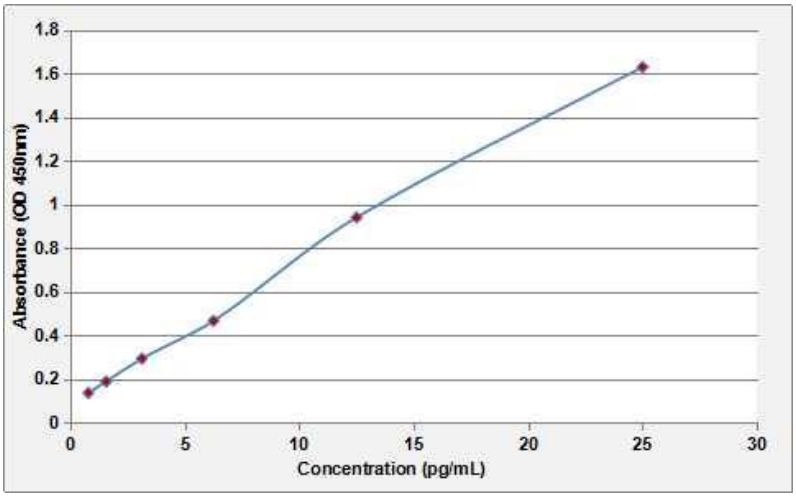
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

11. Typical Expected Data

11.1 Reproducibility

The mean intra-assay and mean inter-assay coefficient of variation has been calculated to be 7.9% and 9.5% respectively.

11.2 Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



Standard	IL-12p70 (pg/mL)	Mean OD (450nm)	CV (%)
1	25	1.628	4.3
2	12.5	0.939	1.7
3	6.25	0.465	8.8
4	3.12	0.292	9.2
5	1.56	0.187	1.9
6	0.78	0.135	0.0
Zero	0	0.076	-

11.3 Linearity

A pooled human serum containing 25 pg/ml of measured IL-12 was serially diluted in standard buffer diluent over the range of the assay. Linear regression of samples versus the expected concentration yielded a quote slope of 0.99.

11.4 Recovery

Recovery of IL-12 added to pooled normal serum was 89% for IL-12 concentration ranging from 25 to 1.56 pg/ml.

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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