Human IL-12 (p70) High Sensitivity ELISA Kit

Enzyme-linked immunosorbent assay (ELISA) for quantitative detection of human IL-12p70

Catalog Number BMS238-2HS

Pub. No. MAN0030003 Rev. A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Human IL-12 (p70) High Sensitivity ELISA Kit (Cat. No. BMS238-2HS) is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-12p70.

Summary

Interleukin-12 (IL-12) is a pleiotropic cytokine, formerly termed cytotoxic lymphocyte maturation factor (CLMF) or natural killer cell stimulatory factor (NKSF), that is produced primarily by stimulated macrophages. IL-12 was originally identified as a factor produced by human Epstein-Barr Virus-transformed B cell lines. However, IL-12 has since been shown to be a proinflammatory cytokine produced by phagocytic cells, B cells, and other antigen-presenting cells that modulate adaptive immune responses by favoring the generation of T-helper type 1 cells.

IL-12 exerts a variety of biological effects on human T and natural killer cells. Apart from promotion of Th1 development and cytolytic activity, IL-12 also acts as a potent inducer of interferon γ (IFN-γ) production and stimulator of other cytokines from peripheral blood T and NK cells. IFN-γ acts in a positive feedback loop, enhancing the ability of phagocytic cells to produce IL-12 and other proinflammatory cytokines; an important amplifying mechanism in the inflammatory response to infections.

The role of IL-12 in directing development of a Th1 type immune response from naive T cells demonstrates its critical role in regulation of the immune response and strongly suggests its potential usefulness in cancer therapy.

IL-12 is a disulfide-linked heterodimeric cytokine composed of a 35 kDa light chain (p35) and a 40 kDa heavy chain (p40), resulting in the only biologically active 70 kDa (p70) form of IL-12. The p40 subunit can also form a homodimer which has been shown to be able to bind the IL-12 receptor and thus, acts as an IL-12 antagonist. Additionally, the p40 subunit has been found to be expressed in high excess over p70.

Various studies have shown that IL-12 plays a critical role in the pathogenesis of several immune-mediated inflammatory diseases.

For literature updates, go to thermofisher.com.

Principles of the test

An anti-human monoclonal antibody (mAb) that is specific to human IL-12p70 is adsorbed onto microwells.

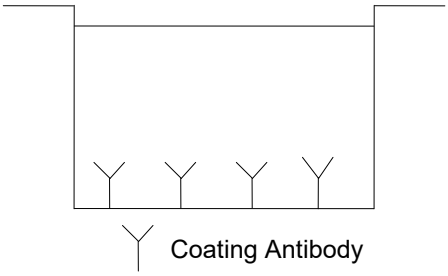

Fig. 1 Coated microwell

Human IL-12p70 that is present in the sample or standard binds to the immobilized (capture) antibodies. A biotin-conjugated anti-human IL-12p70 antibody (Biotin-Conjugate) is then added, which binds to the human IL-12p70 that was captured by the first antibody.

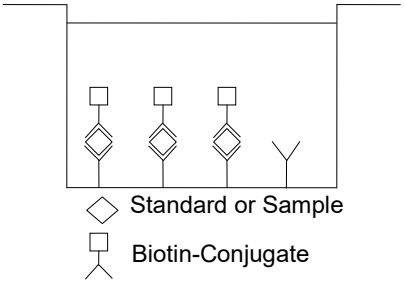

Fig. 2 First incubation

Following incubation, unbound Biotin-Conjugate is removed during a wash step, then Streptavidin-HRP is added, which binds to the Biotin-Conjugate.

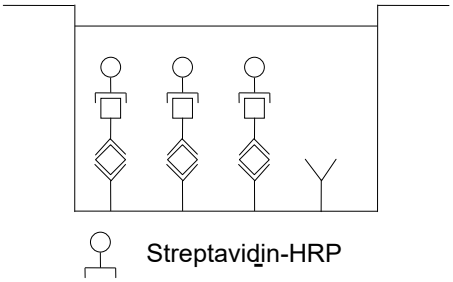

Fig. 3 Second incubation

Following incubation, unbound Streptavidin-HRP is removed during a wash step, then Amplification Reagent I (biotin-anti-HRP) is added.

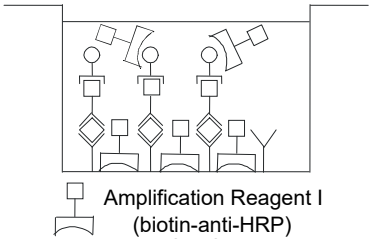

Fig. 4 Third incubation

Following incubation, unbound Amplification Reagent I is removed during a wash step, then Amplification Reagent II (streptavidin-HRP) is added.

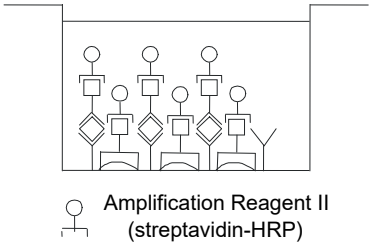

Fig. 5 Fourth incubation

Following incubation, unbound Amplification Reagent II is removed during a wash step, then the Substrate Solution is added that reacts with HRP.

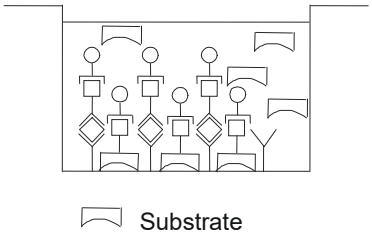

Fig. 6 Fifth incubation

A colored product is formed in proportion to the amount of human IL-12p70 present in the sample or standard. The reaction is terminated by the addition of an acid, then absorbance is measured at 450 nm. A standard curve, prepared from 7 human IL-12p70 standard dilutions, allows the determination of human IL-12p70 concentration in the sample.

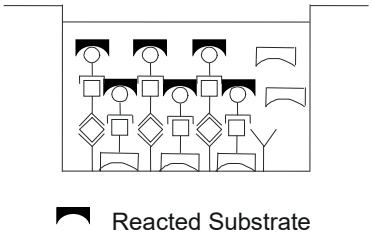

Fig. 7 Stop reaction

Principle of the amplification reaction

Amplification Reagent I contains a biotin-conjugated anti-HRP antibody that reacts with the streptavidin-HRP that is added after the first incubation (see Figure 3 and Figure 4). Following incubation, unbound biotin-conjugated anti-HRP antibody is removed during a wash step. Amplification Reagent II, containing streptavidin-HRP then binds to the biotin sites of the Amplification Reagent I (see Figure 5).

Reagents provided

- 1 aluminum pouch with a microwell plate (12 strips of 8 wells each) pre-coated with mAb to human IL-12p70
- 1 vial (70 µL) of Biotin-Conjugate (biotin-conjugated anti-human IL-12p70 mAb)
- 1 vial (150 µL) of Streptavidin-HRP
- 2 vials of Human IL-12p70 Standard, lyophilized (400 pg/mL upon reconstitution)
- 1 vial (25 mL) of Sample Diluent
- 1 vial (5 mL) of Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20 and 10% BSA)
- 1 vial (120 µL) of Amplification Reagent I
- 1 vial (120 µL) of Amplification Reagent II
- 2 bottles (50 mL) of Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)
- 1 vial (15 mL) of Substrate Solution
- 1 vial (15 mL) of Stop Solution (1M Phosphoric acid)
- 8 adhesive films

Storage instructions – ELISA kit

- Store kit reagents at 2° to 8°C.
- Immediately after use, return remaining reagents to cold storage (2°C to 8°C). See the expiration date on the package.
- Expiry of the kit components can be guaranteed only if the components are stored properly, and if, in the case of repeated use of one component, the reagent is not contaminated by the first handling.

Sample collection and storage

- Cell culture supernatant, serum, and plasma (EDTA and citrate) were tested with this assay. Other biological samples might be suitable for use in the assay.
- Remove serum or plasma from the clot (or cells) as soon as possible after clotting and separation.
- Clarify samples that contain a visible precipitate before use. Do not use grossly hemolyzed or lipemic samples.
- Aliquot, then freeze samples at –20°C to avoid loss of bioactive human IL-12p70. If samples are to be run within 24 hours, they can be stored at 2°C to 8°C (see “Sample stability” on page 7).
- Thaw frozen samples slowly at room temperature, then gently mix before use. Avoid repeated freeze-thaw cycles.

Required materials not supplied

- 5-mL and 10-mL graduated pipettes
- 5-µL to 1,000-µL adjustable single channel micropipettes with disposable tips
- 50-µL to 300-µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and cylinders for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wavelength)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Procedural guidelines

- All reagents should be considered as potentially hazardous. This product should be handled only by those persons who have been trained in laboratory techniques and should be used in accordance with the principles of good laboratory practice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Wear rubber or disposable latex gloves while handling kit reagents or samples.
- Avoid contact of Substrate Solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- Use disposable pipette tips and/or pipettes to avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Do not expose the HRP-Conjugate to an acid to prevent inactivation.
- Use glass-distilled water or deionized water for reagent preparation.
- Equilibrate the Substrate Solution to room temperature before use.
- Decontaminate and dispose of samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid waste that does not contain an acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste that contains an acid must be neutralized before the addition of sodium hypochlorite.
- Once the test has been started, complete all steps without interruption.
- Absorbance is a function of incubation time and temperature. Before starting the assay, ensure that all reagents are ready, caps are removed, and all needed wells are secured in a holder. This will ensure equal elapsed time for each pipetting step.
- As a general rule, the enzymatic reaction is linearly proportional to time and temperature.
- Adhere to the incubation times as stated in this user guide.
- We recommend running calibrators, controls, and samples in duplicate (at a minimum).
- Establish a calibrator curve for every test run.

Guidelines for sample dilutions

- Do not predilute serum or plasma samples.
- The optimal dilution factor for cell culture supernatants must be determined empirically, for each individual sample. To accurately measure cell culture supernatants with very high expected concentrations of IL-12p70, a high dilution factor (e.g., up to 1:2,000) may be required. In such cases, predilute samples in the appropriate culture medium. The final dilution should be performed in Sample Diluent as described in the procedure.
- For unknown cell culture samples, we recommend running undiluted and prediluted (1:20 to 1:50) samples in parallel to cover a wider range of concentrations in one assay.

Before you begin

- Equilibrate the buffer concentrates to room temperature, then dilute before use (see “Prepare reagents” on page 3).
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

Prepare reagents

Prepare Wash Buffer (1x)

1. Transfer the entire contents (50 mL) of the Wash Buffer Concentrate (20x) to a clean 1,000-mL graduated cylinder, then bring to a final volume of 1,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.

   Wash Buffer (1x) can also be prepared as needed according to the following table.

Number of strips	Wash Buffer Concentrate (20x)	Distilled water
1–6	25 mL	475 mL
1–12	50 mL	950 mL

2. Transfer to a clean wash bottle.

Store Wash Buffer (1x) at 2°C to 25°C for up to 30 days.

Prepare Assay Buffer (1x)

Transfer the entire contents (5 mL) of the Assay Buffer Concentrate (20x) to a clean 100-mL graduated cylinder, then bring to a final volume of 100 mL with distilled water. Mix gently to avoid foaming.

Assay Buffer (1x) can also be prepared as needed according to the following table.

Number of strips	Assay Buffer Concentrate (20x)	Distilled water
1–6	2.5 mL	47.5 mL
1–12	5.0 mL	95.0 mL

Store Assay Buffer (1x) at 2°C to 8°C for up to 30 days.

Prepare Biotin-Conjugate

Note: Prepare Biotin-Conjugate within 30 minutes of usage.

Dilute the concentrated Biotin-Conjugate solution 1:100 with Sample Diluent in a clean plastic tube according to the following table.

Number of strips	Biotin-Conjugate (concentrated solution)	Sample Diluent
1–6	0.03 mL	2.97 mL
1–12	0.06 mL	5.94 mL

Prepare Streptavidin-HRP

Note: Prepare Streptavidin-HRP within 30 minutes of usage.

Dilute the Streptavidin-HRP 1:100 with Assay Buffer (1x) in a clean plastic tube according to the following table.

Number of strips	Streptavidin-HRP	Assay Buffer (1x)
1–6	0.06 mL	5.94 mL
1–12	0.12 mL	11.88 mL

Prepare Human IL-12p70 Standard

Prepare fresh standard on each day of use.

1. Reconstitute Human IL-12p70 Standard using distilled water. The reconstitution volume is stated on the label.

   Note: The concentration of the reconstituted standard is 400 pg/mL.
2. Swirl or mix gently to ensure complete and homogeneous solubilization.
3. Allow the standard to reconstitute for 10–30 minutes, then mix well.
4. In a clean plastic test tube, dilute the reconstituted standard 1:20 with Sample Diluent just before use. For example, combine 50 µL of reconstituted standard with 950 µL of Sample Diluent. Shake gently to mix (concentration of standard = 20.00 pg/mL).
5. Proceed to prepare standard dilutions in tubes (see "Prepare Human IL-12p70 Standard dilutions in tubes" on page 4) or directly in the microwell plate (see "Perform the ELISA protocol" on page 4).

After use, any remaining standard cannot be stored and must be discarded.

Prepare Human IL-12p70 Standard dilutions in tubes

Note: For preparation of Human IL-12p70 Standard dilutions in a microwell plate, see "Perform the ELISA protocol" on page 4.

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 2-fold serial dilutions for the standard curve. Add 225 µL of Sample Diluent into each tube.
3. Add 225 µL of diluted standard (concentration = 20.00 pg/mL) into the first tube, labeled S1, then mix (concentration of standard 1 = 10.00 pg/mL).
4. Add 225 µL of this dilution into the second tube, labeled S2, then mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times to create the points of the standard curve (see Figure 8).

   Sample Diluent serves as a blank.

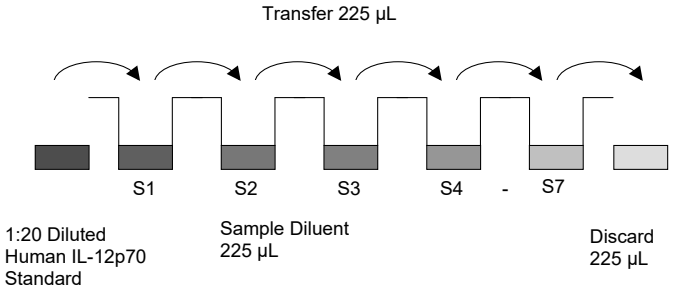

Fig. 8 Dilute standards - tubes

Prepare Amplification Reagent I

Prepare Amplification Reagent I immediately before use.

Dilute Amplification Reagent I in Assay Buffer as indicated in the Certificate of Analysis.

After use, any remaining prediluted Amplification Reagent I cannot be stored and must be discarded.

Prepare Amplification Reagent II

Prepare Amplification Reagent II immediately before use.

Dilute Amplification Reagent II in Assay Buffer as indicated in the Certificate of Analysis.

After use, any remaining prediluted Amplification Reagent II cannot be stored and must be discarded.

Perform the ELISA protocol

IMPORTANT!

- This ELISA is a high-sensitivity system. Follow this procedure as described (preparation of solutions, step sequence, washing procedures, and incubation time) for optimal test performance.
- Prepare amplification reagents immediately before application on the plate!
- Shaking is required for optimal test performance.
- Protect the microwell plate from light during incubation steps.

1. Prepare the samples. See "Guidelines for sample dilutions" on page 3.
2. Determine the number of microwell strips that are required to test the desired number of samples, plus the appropriate number of blanks and standards. Assay each sample, standard, blank, and optional control sample in duplicate.

   Return any unused microwell strips to the provided foil bag with desiccant pack, then store tightly sealed at 2°C to 8°C.
3. Wash the microwell strips twice with exactly 400 µL of Wash Buffer (1x) per well, thoroughly aspirating between washes. Allow the Wash Buffer to sit in the wells for 10–15 seconds before aspiration.

   IMPORTANT! Do not scratch the inner surface of the microwells.

   Note: To ensure optimal test performance, we highly recommend soaking between each wash.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Do not allow wells to dry.
4. Prepare standard dilutions in the microwell plate.

   Note: Alternatively, the standard dilutions can be prepared in tubes. See "Prepare Human IL-12p70 Standard dilutions in tubes" on page 4.

   a. Add 100 µL of Sample Diluent, in duplicate, to all standard wells.
   b. Add 100 µL of the prepared standard (see "Prepare Human IL-12p70 Standard" on page 4, concentration = 20.00 pg/mL), in duplicate, to wells A1 and A2 (see Table 1).
   c. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 10.00 pg/mL), then transfer 100 µL to wells B1 and B2, respectively (see Figure 9). Do not scratch the inner surface of the microwells.

d. Repeat the above procedure 5 times, creating two rows of Human IL-12p70 Standard dilutions ranging from 10.00 pg/mL to 0.16 pg/mL. Discard 100 µL of the contents from the last microwells used (G1 and G2).

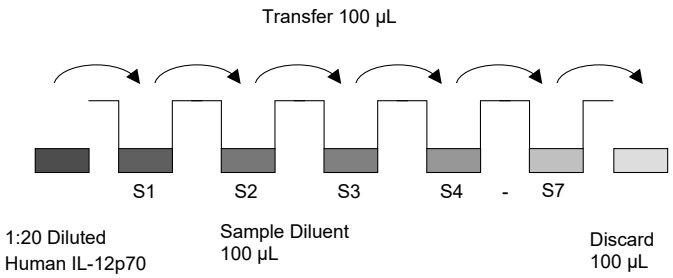

Fig. 9 Standard dilutions in microwell plate

If the standard dilutions were prepared in tubes, transfer 100 µL of each standard dilution (S1 – S7), in duplicate, into the standard wells according to Table 1.

Table 1 Example: Arrangement of blanks, standards, and samples in the microwell strips

	1	2	3	4
A	Standard 1 10.00 pg/mL	Standard 1 10.00 pg/mL	Sample 1	Sample 1
B	Standard 2 5.00 pg/mL	Standard 2 5.00 pg/mL	Sample 2	Sample 2
C	Standard 3 2.50 pg/mL	Standard 3 2.50 pg/mL	Sample 3	Sample 3
D	Standard 4 1.25 pg/mL	Standard 4 1.25 pg/mL	Sample 4	Sample 4
E	Standard 5 0.63 pg/mL	Standard 5 0.63 pg/mL	Sample 5	Sample 5
F	Standard 6 0.31 pg/mL	Standard 6 0.31 pg/mL	Sample 6	Sample 6
G	Standard 7 0.16 pg/mL	Standard 7 0.16 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

5. Add 100 µL of Sample Diluent, in duplicate, to the blank wells.
6. Add 50 µL of Sample Diluent to the sample wells.
7. Add 50 µL of each sample, in duplicate, to the sample wells.
8. Prepare Biotin-Conjugate (see “Prepare Biotin-Conjugate” on page 3).
9. Add 50 µL of Biotin-Conjugate to all wells.
10. Cover with an adhesive film, then incubate for 2 hours at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
11. Prepare the Streptavidin-HRP (see “Prepare Streptavidin-HRP” on page 4).
12. Remove the adhesive film, then empty the wells. Wash the microwell strips 6 times as previously described (see step 3), then proceed immediately to the next step.
13. Add 100 µL of prepared Streptavidin-HRP to all wells, including the blank wells.
14. Cover with an adhesive film, then incubate for 1 hour at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
15. Prepare Amplification Reagent I immediately before use (see “Prepare Amplification Reagent I” on page 4).
16. Remove the adhesive film, then empty the wells. Wash the microwell strips 6 times as previously described (see step 3), then immediately proceed to the next step.
17. Add 100 µL of prepared Amplification Reagent I to all wells, including the blank wells.
18. Cover with an adhesive film, then incubate for exactly 15 minutes at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
19. Prepare Amplification Reagent II immediately before use (see “Prepare Amplification Reagent II” on page 4).
20. Remove the adhesive film, then empty the wells. Wash the microwell strips 6 times as previously described (see step 3), then immediately proceed to the next step.
21. Add 100 µL of prepared Amplification Reagent II to all wells, including the blank wells.
22. Cover with an adhesive film, then incubate for exactly 30 minutes at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
23. Remove the adhesive film, then empty the wells. Wash the microwell strips 6 times as previously described (see step 3), then proceed immediately to the next step.
24. Add 100 µL of Substrate Solution to all wells.
25. Incubate the microwell strips for about 10-20 minutes at room temperature (18–25°C). Avoid direct exposure to intense light.
26. Quickly add 100 µL of Stop Solution to each well to stop the enzyme reaction, then read the results immediately or within one hour if the microwell strips are stored at 2°C to 8°C in the dark. It is important to add the Stop Solution quickly and uniformly throughout the microwell plate to completely inactivate the enzyme.
27. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optionally, 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions, using the blank wells. Determine the absorbance of both the samples and the standards.

    If the instructions in this protocol have been followed and samples have been diluted 1:2 (50 µL of sample + 50 µL of Sample Diluent), multiply each concentration by 2.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-12p70 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human IL-12p70 for each sample, first find the mean absorbance value on the ordinate, then extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa, then read the corresponding human IL-12p70 concentration.
- Multiply each concentration read from the standard curve by the sample dilution factor. If the instructions in this protocol have been followed and samples have been diluted 1:2 (50 µL of sample + 50 µL of Sample Diluent), multiply each concentration by 2.
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human IL-12p70 levels. Such samples require further external predilution with Sample Diluent according to expected human IL-12p70 values to precisely quantitate the actual human IL-12p70 level.
- It is suggested that each testing facility establishes a control sample of known human IL-12p70 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

A representative standard curve is shown in Figure 10.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Note: The OD values of the standard curve may vary according to the conditions or assay performance (e.g., operator, pipetting

technique, washing technique, or temperature effects). In addition, the shelf-life of the kit can affect enzymatic activity and thus, color intensity. Values measured, however, are still valid.

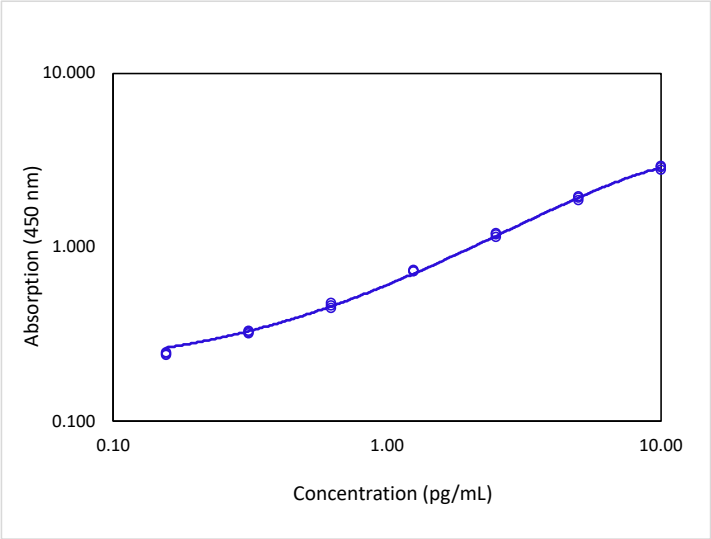

Fig. 10 Representative standard curve for the Human IL-12 (p70) High Sensitivity ELISA Kit

Human IL-12p70 Standard was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the Human IL-12 (p70) High Sensitivity ELISA Kit

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human IL-12p70 (pg/mL)	OD at 450 nm	Mean OD at 450 nm	C.V. (%)
1	10.00	2.942 2.781	2.862	4.0
2	5.00	1.947 1.858	1.903	3.0
3	2.50	1.199 1.147	1.173	3.0
4	1.25	0.734 0.719	0.727	1.0
5	0.63	0.479 0.444	0.462	5.0
6	0.31	0.328 0.317	0.323	2.0
7	0.16	0.248 0.241	0.245	2.0
Blank	0.15	0.159 0.150	0.154	4.0

Limitations

- Since exact conditions may vary between assays, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents, or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred. Reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false-positive or false-negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle, and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false-positive and false-negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Performance characteristics

Sensitivity

The limit of detection of human IL-12p70 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.1 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of human IL-12p70. Two standard curves were run on each plate. Data below show the mean human IL-12p70 concentration and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 6.7%.

Table 3 The mean human IL-12p70 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean concentration (pg/mL)	Coefficient of variation (%)
1	1	17.1	1.5
	2	17.9	12.3
	3	16.6	7.3
2	1	11.4	3.6
	2	11.6	5.3
	3	10.2	14.8
3	1	8.4	4.1
	2	7.9	4.5
	3	8.1	3.2
4	1	5.8	5.5
	2	6.0	3.2
	3	6.0	4.6
5	1	3.1	16.4
	2	3.2	4.6
	3	3.3	5.9
6	1	2.2	11.8
	2	1.9	4.1
	3	2.0	5.4
7	1	2.8	7.5
	2	2.7	3.5
	3	2.9	7.6
8	1	0.5	10.9
	2	0.5	5.9
	3	0.6	7.3

Inter-assay

Assay-to-assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of human IL-12p70. Two standard curves were run on each plate. Data below show the mean human IL-12p70 concentration and the coefficient of variation calculated on 18 determinations of each

sample. The calculated overall inter-assay coefficient of variation was 5.4%.

Table 4 The mean human IL-12p70 concentration and the coefficient of variation for each sample

Sample	Mean concentration (pg/mL)	Coefficient of variation (%)
1	17.0	4.0
2	11.0	6.7
3	8.0	3.1
4	6.0	1.9
5	3.0	2.2
6	2.0	7.0
7	3.0	4.2
8	1.0	14.2

Spike recovery

The spike recovery was evaluated by spiking 3 levels of human IL-12p70 into serum, plasma (EDTA and citrate), and cell culture supernatant. Recoveries were determined with 4 replicates each. The amount of endogenous human IL-12p70 in unspiked samples was subtracted from the spike values.

Sample matrix	Spike high (%)	Spike medium (%)	Spike low (%)
Serum	96%	85%	82%
Plasma (EDTA)	88%	87%	78%
Plasma (citrate)	103%	106%	124%
Cell culture supernatant	97%	106%	106%

Dilution parallelism

Serum, plasma (EDTA and citrate), and cell culture supernatant samples with different levels of human IL-12p70 were analyzed at serial 2-fold dilutions with 4 replicates each.

Sample matrix	Recovery of expected values	
	Range (%)	Mean (%)
Serum	1:4 1:8 1:16	97 91 89
Plasma (EDTA)	1:4 1:8 1:16	105 103 91
Plasma (citrate)	1:4 1:8 1:16	92 83 79
Cell culture supernatant	1:4 1:8 1:16	100 99 120

Sample stability

Freeze-thaw stability

Aliquots of serum and cell culture supernatant samples (spiked or unspiked) were stored at –20°C and thawed 5 times, then human IL-12p70 levels determined. There was no significant loss of human IL-12p70 immunoreactivity detected up to 3 cycles of freezing and thawing. Further freeze-thaw cycles gave rise to approximately 20% loss of human IL-12p70 immunoreactivity.

Storage stability

Aliquots of serum and cell culture supernatant samples (spiked or unspiked) were stored at –20°C, 2–8°C, room temperature, and at 37°C, then human IL-12p70 level determined after 24 hours. There was no significant loss of human IL-12p70 immunoreactivity detected during storage at –20°C, 2–8°C, and room temperature.

A significant loss of human IL-12p70 immunoreactivity (20%) was detected during storage at 37°C after 24 hours.

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IL-12p70 positive serum. No cross-reactivity was detected.

Expected values

No detectable human IL-12p70 levels were found in healthy blood donors. Elevated human IL-12p70 levels depend on the type of immunological disorder and the severity of disease.

Calibration

The immunoassay is calibrated with highly purified recombinant human IL-12p70, which has been evaluated against the international Reference Standard, NIBSC 95/544, and has been shown to be equivalent.

NIBSC 95/544 is quantitated in International Units (IU); 1IU corresponds to 100 pg of human IL-12p70.

Reagent preparation summary

Prepare Wash Buffer (1x)

Add Wash Buffer Concentrate 20x (50 mL) to 950 mL of distilled water.

Number of strips	Wash Buffer Concentrate (20x)	Distilled water
1–6	25 mL	475 mL
1–12	50 mL	950 mL

Prepare Assay Buffer (1x)

Add Assay Buffer Concentrate 20x (5 mL) to 95 mL of distilled water.

Number of strips	Assay Buffer Concentrate (20x)	Distilled water
1–6	2.5 mL	47.5 mL
1–12	5.0 mL	95.0 mL

Prepare Biotin-Conjugate

Make a 1:100 dilution of Biotin-Conjugate in Sample Diluent:

Number of strips	Biotin-Conjugate	Sample Diluent
1–6	0.03 mL	2.97 mL
1–12	0.06 mL	5.94 mL

Prepare Streptavidin-HRP

Make a 1:100 dilution of Streptavidin-HRP in Assay Buffer (1x):

Number of strips	Streptavidin-HRP	Assay Buffer (1x)
1–6	0.06 mL	5.94 mL
1–12	0.12 mL	11.88 mL

Prepare Human IL-12p70 Standard

Reconstitute Human IL-12p70 Standard using distilled water. The reconstitution volume is stated on the label.

Dilute the reconstituted Human IL-12p70 Standard 1:20 with Sample Diluent just before use.

Prepare Amplification Reagent I

Dilute Amplification Reagent I in Assay Buffer immediately before use, as indicated in the Certificate of Analysis.

Prepare Amplification Reagent II

Dilute Amplification Reagent II in Assay Buffer immediately before use, as indicated in the Certificate of Analysis.

ELISA protocol summary

Note:

- It is extremely important to wash the wells properly for optimal test performance.
- Prepare amplification reagents immediately before use.
- Shaking is required for optimal test performance.
- Protect the microwell plate from light during incubation steps.

1. Determine the number of microwell strips required.
2. Wash the microwell strips twice with Wash Buffer (1x).
3. Perform standard dilutions on the microwell plate: Add 100 µL of Sample Diluent, in duplicate, to all standard wells. Add 100 µL of prepared standard into the first wells, then create standard dilutions by transferring 100 µL from well-to-well. Discard 100 µL from the last wells.

   Alternatively, if the standard dilutions were prepared in tubes, transfer 100 µL of each standard dilution, in duplicate, to the appropriate wells of the microwell plate.
4. Add 100 µL of Sample Diluent, in duplicate, to the blank wells.
5. Add 50 µL of Sample Diluent to the sample wells.
6. Add 50 µL of sample, in duplicate, to the designated sample wells.
7. Prepare the Biotin-Conjugate.
8. Add 50 µL of Biotin-Conjugate to all wells.
9. Cover the microwell strips, then incubate for 2 hours at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
10. Prepare the Streptavidin-HRP.
11. Empty, then wash the microwell strips 6 times with Wash Buffer (1x).
12. Add 100 µL of prepared Streptavidin-HRP to all wells.
13. Cover the microwell strips, then incubate for 1 hour at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
14. Prepare Amplification Reagent I immediately before use.
15. Empty, then wash the microwell strips 6 times with Wash Buffer (1x).
16. Add 100 µL of prepared Amplification Reagent I to all wells.
17. Cover the microwell strips, then incubate for exactly 15 minutes at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
18. Prepare Amplification Reagent II immediately before use.
19. Empty, then wash the microwell strips 6 times with Wash Buffer (1x).
20. Add 100 µL of prepared Amplification Reagent II to all wells.
21. Cover the microwell strips, then incubate for exactly 30 minutes at room temperature (18–25°C) on a microplate shaker. Shaking is required for optimal test performance.
22. Empty, then wash the microwell strips 6 times with Wash Buffer (1x).
23. Add 100 µL of Substrate Solution to all wells.
24. Incubate the microwell strips for about 10-20 minutes at room temperature (18–25°C).
25. Add 100 µL of Stop Solution to all wells.
26. Blank the microwell reader, then measure the color intensity at 450 nm.

    If the instructions in this protocol have been followed and samples have been diluted 1:2 (50 µL of sample + 50 µL of Sample Diluent), multiply each concentration by 2.

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Revision	Date	Description
A.0	18 September 2023	New document for the Human IL-12 (p70) High Sensitivity ELISA Kit.

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

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