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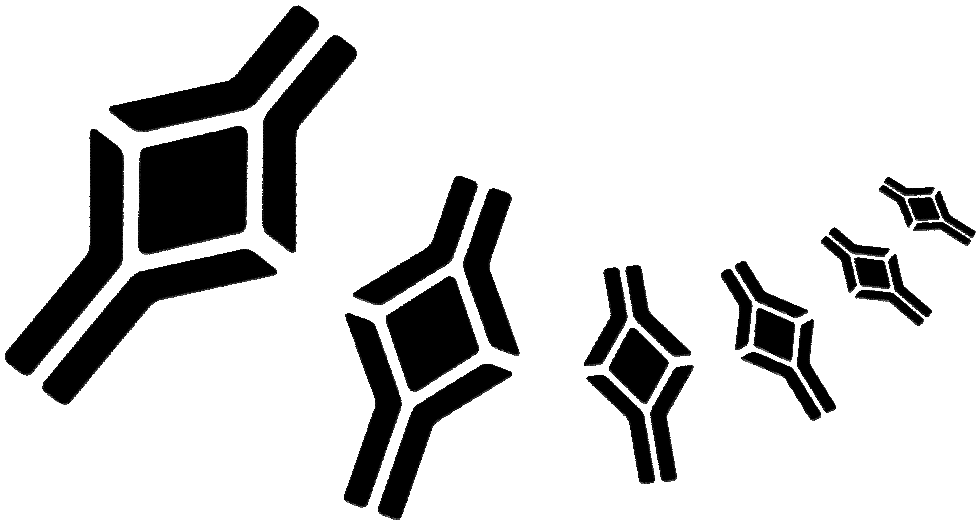


Mouse INTERLEUKIN-12 p70 ELISA

Product Data Sheet

Cat. No.: RBMS6004R

For Research Use Only

CONTENTS

This kit is manufactured by:
BioVendor – Laboratorní medicína, a.s.

Use only the current version of Product Data Sheet enclosed with the kit!

1 INTENDED USE

The mouse IL-12 p70 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of murine IL-12 p70 in serum, cell culture supernatants, plasma, or other body fluids. **The mouse IL-12 p70 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2 SUMMARY

Interleukin-12 (IL-12) is a pleiotropic cytokine, formerly termed cytotoxic lymphocyte maturation factor (CLMF) or natural killer cell stimulatory factor (NKSF) (5, 9, 10), which is produced primarily by stimulated macrophages. IL-12 has been shown to be a proinflammatory cytokine produced by phagocytic cells (23), B cells (5, 9), and other antigen - presenting cells that modulate adaptive immune responses by favoring the generation of T-helper type 1 cells (7).

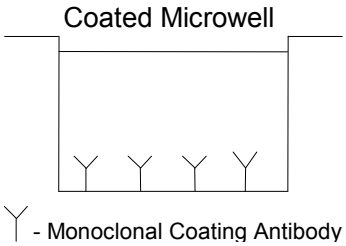
IL-12 exerts a variety of biological effects on T and natural killer cells. Apart from promotion of Th1 development and its ability to promote cytolytic activity it mediates some of its physiological activities by acting as a potent inducer of interferon (IFN) gamma production and the stimulation of other cytokines from peripheral blood T and NK cells, (12, 13). IFN-gamma then enhances the ability of the phagocytic cells to produce IL-12 and other proinflammatory cytokines. Thus, IL-12 induced IFN-gamma acts in a positive feedback loop that represents an important amplifying mechanism in the inflammatory response to infections (7).

Its role 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 (10). IL-12 is a disulfide-linked heterodimeric cytokine composed of a 35kDa light chain (p35) and a 40kDa heavy chain (p40) resulting in the only biologically active 70kDa (p70) form of IL-12 (3). 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 (1, 4). Additionally, the p40 subunit has been found to be expressed in a high excess over p70.

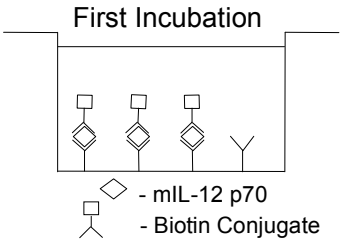
The critical role of IL-12 in several pathogeneses has been shown.

3 PRINCIPLES OF THE TEST

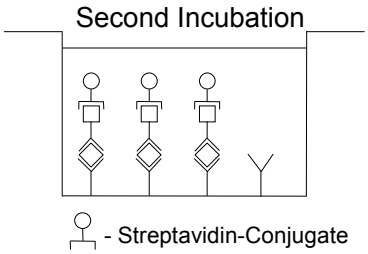
An anti-mIL-12 p70 monoclonal coating antibody is adsorbed onto microwells.



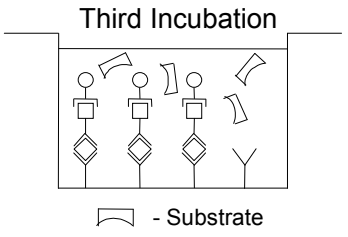
mIL-12 p70 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-mIL-12 p70 antibody is added and binds to mIL-12 p70 captured by the first antibody.

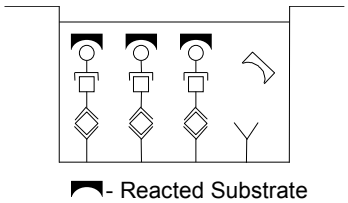


Following incubation unbound biotin conjugated anti-mIL-12 p70 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-mIL-12 p70. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.



A coloured product is formed in proportion to the amount of mIL-12 p70 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven mIL-12 p70 standard dilutions and mIL-12 p70 sample concentration determined.





4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with Monoclonal Antibody (rat) to murine IL-12 p70

1 vial (100 µl) **Biotin-Conjugate** anti-mIL-12 p70 monoclonal antibody[1,2)]

1 vial (150 µl) **Streptavidin-HRP**

2 vials **mIL-12 p70 Standard,** lyophilized, 2000 pg/ml upon reconstitution

1 vial (5 ml) **Assay Buffer Concentrate** 20x
(PBS with 1 % Tween 20 and 10 % BSA)

1 bottle (50 ml) **Wash Buffer Concentrate** 20x
(PBS with 1% Tween 20)

1 bottle (12 ml) **Sample Diluent**

1 vial (7 ml) **Substrate Solution I** (tetramethyl-benzidine)

1 vial (7 ml) **Substrate Solution II**
(0.02 % buffered hydrogen peroxide)

1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml) ***Blue-Dye***

1 vial (0.4 ml) ***Red-Dye***

1 vial (0.4 ml) ***Green-Dye***

4 adhesive **Plate Covers**

Reagent Labels

5 STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 SPECIMEN COLLECTION

Cell culture supernatants, murine serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive mIL-12 p70. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to respective chapter.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes

- 10 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, cylinders necessary for preparation of reagents

- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)

- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

- Glass-distilled or deionized water

- Statistical calculator with program to perform linear regression analysis

8 PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.

- 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 pipette 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 specimens.

- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.

- Reagents containing thimerosal as preservative may be toxic if ingested.

- Avoid contact of substrate solutions with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.

- Exposure to acids will inactivate the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.

- Substrate solutions must be at room temperature prior to use.

- Decontaminate and dispose specimens 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 wastes not containing 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 containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 PREPARATION OF REAGENTS

A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

B. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer** Concentrate (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table.

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** with **Assay Buffer** in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Preparation of mIL-12 p70 Standard

Reconstitute mIL-12 p70 **Standard** by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard. Mix gently to ensure complete solubilization. Store reconstituted Standard promptly at –20°C. Discard after one week.

E. Preparation of Streptavidin-HRP

Make a 1:200 dilution of the concentrated **Streptavidin-HRP** solution in **Assay Buffer** as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1 - 6	0.03	6
1 - 12	0.06	12

F. TMB Substrate Solution

Using clean pipettes and containers known to be metal free, dispense an equal volume of **Substrate Solution I** into **Substrate Solution II** and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue colour present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation.

Substrate preparation according to assay size:

Number of Strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

G. Addition of Colour-giving Dyes

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.
Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye, Red-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent:

Before sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 ml Diluent	20 µl ***Blue-Dye***
12 ml Diluent	48 µl ***Blue-Dye***

2. Biotin-Conjugate:

Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of Biotin-conjugate.

3 ml Assay Buffer	30 µl ***Green-Dye***
6 ml Assay Buffer	60 µl ***Green-Dye***

3. Streptavidin-HRP:

Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

6 ml Assay Buffer	24 µl ***Red-Dye***
12 ml Assay Buffer	48 µl ***Red-Dye***

10 TEST PROTOCOL

a. Prepare reagents immediately before use and mix them thoroughly without foaming.

b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (rat) to murine IL-12 p70 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. Wash the microwell strips twice with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µl of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (Refer to preparation of reagents) **mIL-12 p70 Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of mIL-12 p70 standard dilutions ranging from 1000 to 15.6 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of mIL-12 p70 standard dilutions:

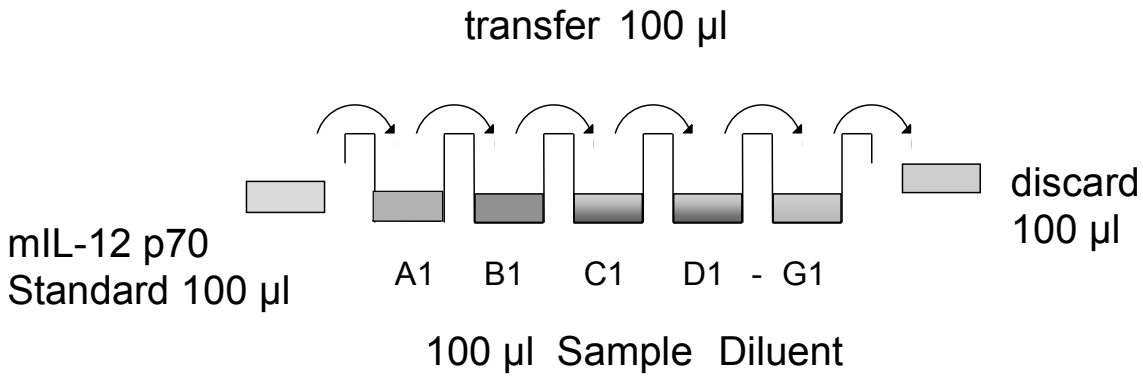


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (1000 pg/ml)	Standard 1 (1000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (500 pg/ml)	Standard 2 (500 pg/ml)	Sample 2	Sample 2
C	Standard 3 (250 pg/ml)	Standard 3 (250 pg/ml)	Sample 3	Sample 3
D	Standard 4 (125 pg/ml)	Standard 4 (125 pg/ml)	Sample 4	Sample 4
E	Standard 5 (62.5 pg/ml)	Standard 5 (62.5 pg/ml)	Sample 5	Sample 5
F	Standard 6 (31.3 pg/ml)	Standard 6 (31.3 pg/ml)	Sample 6	Sample 6
G	Standard 7 (15.6 pg/ml)	Standard 7 (15.6 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µl of **Sample Diluent,** in duplicate, to the blank wells.

f. Add 50 µl of **Sample Diluent** to the sample wells.

g. Add 50 µl of each **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate**. (Refer to preparation of reagents)

i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 2 hours, if available on a microplate shaker set at 200 rpm.

k. Prepare **Streptavidin-HRP**. (Refer to preparation of reagents)

l. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker at 200 rpm.

o. Prepare **TMB Substrate Solution** a few minutes prior to use. (Refer to preparation of reagents).

p. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

q. Pipette 100 µl of mixed **TMB Substrate Solution** to all wells, including the blank wells.

r. Incubate the microwell strips at room temperature (18° to 25°C) for about **10 minutes**, if available on a microplate shaker at 200 rpm. Avoid direct exposure to intense light. The point at which the substrate reaction need to be stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance only up to 2.0 O.D. **Therefore the colour development within individual microwells must be watched by the person running the assay. The substrate reaction must be stopped before positive wells are no longer properly recordable.**

s. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

t. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the mIL-12 p70 standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the mIL-12 p70 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating mIL-12 p70 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding mIL-12 p70 concentration.

  If samples have been diluted according to the instructions given in this manual 1:2, the concentration read from the standard curve must be multiplied by the respective dilution factor (x2).

  Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low mIL-12 p70 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual mIL-12 p70 level.

  It is suggested that each testing facility establishes a control sample of known mIL-12 p70 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for mIL-12 p70 ELISA. mIL-12 p70 was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations.
Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Typical data using the mIL-12 p70 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	mIL-12 p70 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	1000	2.009 1.979	1.994	1.1
2	500	1.166 1.182	1.174	1.0
3	250	0.641 0.657	0.649	1.7
4	125	0.345 0.344	0.345	0.2
5	62.5	0.191 0.212	0.202	7.4
6	31.3	0.116 0.125	0.121	5.3
7	15.6	0.077 0.078	0.078	0.9
Blank	0	0.044 0.040	0.042	6.7

12 LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either 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. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

13 PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of mIL-12 p70 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be < 10 pg/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of IL-12 p70. Two standard curves were run on each plate. Data below show the mean IL-12 p70 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 8.3%.

Positive Sample	Experiment	mIL-12 p70 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	630	9
	2	495	10
	3	600	6
2	1	365	9
	2	352	5
	3	362	3
3	1	475	11
	2	475	5
	3	397	3
4	1	850	8
	2	670	12
	3	858	10
5	1	412	11
	2	330	7
	3	376	8
6	1	219	9
	2	165	7
	3	173	6
7	1	135	14
	2	105	10
	3	126	10

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of mIL-12 p70. Two standard curves were run on each plate. Data below show the mean mIL-12 p70 concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 11.0%.

Sample	mIL-12 p70 Concentration (pg/ml)	Coefficient of Variation (%)
1	575	12.3
2	360	1.9
3	449	10.0
4	793	13.4
5	374	11.0
6	186	15.6
7	122	12.5

C. Spiking Recovery

The spiking recovery was evaluated by spiking three levels of mIL-12 p70 into pooled normal murine serum. Recoveries were determined in three independent experiments with 2 replicates each. Observed values ranged from 95 – 118% with an overall mean recovery of 106%.

D. Dilution Linearity

Murine serum spiked with different levels of mIL-12 p70 was assayed at four serial twofold dilutions with 4 replicates each. Recoveries ranged from 87.7% to 117.1% with an overall mean recovery of 101.9%.

mIL-12 p70 Concentration (pg/ml)				
Sample	Dilution	Expected Value	Observed Value	% Recovery of Exp. Value
1	1:2	--	711	--
	1:4	356	385	108.3
	1:8	193	207	107.5
	1:16	104	117	113.1
2	1:2	--	1013	--
	1:4	507	494	97.5
	1:8	247	233	94.1
	1:16	116	118	101.4
3	1:2	--	467	--
	1:4	234	208	89.0
	1:8	104	108	103.8
	1:16	54	56	103.8
4	1:2	--	545	--
	1:4	272	239	87.7
	1:8	119	119	99.3
	1:16	59	69	117.1

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of spiked serum were stored frozen at –20°C and thawed up to 5 times, and mIL-12 p70 levels determined. There was no significant loss of IL-12 p70 by freezing and thawing up to 5 times.

b. Storage Stability

Aliquots of spiked serum were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the mIL-12 p70 level determined after 72 h. There was no significant loss of mIL-12 p70 immunoreactivity during above storage conditions.

F. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a mIL-12 p70 positive serum. There was no detectable cross reactivity.

G. Expected Serum Values

There are no detectable mIL-12 p70 levels found in healthy mice. Elevated mIL-12 p70 levels depend on the type of immunological disorder.

14 REFERENCES

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15 REAGENT PREPARATION SUMMARY

A. Wash Buffer

Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

B. Assay Buffer

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Biotin-Conjugate

Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Standard

Add distilled water to each vial of lyophilized **mIL-12 p70 Standard** (volume is stated on the label) as needed.

E. Streptavidin-HRP

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1 - 6	0.03	6
1 - 12	0.06	12

F. TMB Substrate Solution

Number of Strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

16 TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells
- Pipette 100 µl reconstituted **mIL-12 p70 Standard** into the first wells and create standard dilutions ranging from 1000 to 15.6 pg/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 50 µl **Sample Diluent** to the sample wells
- Add 50 µl **Sample**, in duplicate, to designated wells
- Prepare Biotin-Conjugate
- Add 50 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature **(18° to 25°C)** on a microplate shaker
- Prepare Streptavidin-HRP
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature **(18° to 25°C)** on a microplate shaker
- Prepare **TMB Substrate Solution** few minutes prior to use
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of mixed **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for 10-20 minutes at room temperature **(18°to 25°C)** on a microplate shaker
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low mIL-12 p70 levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual mIL-12 p70 level.

NOTES

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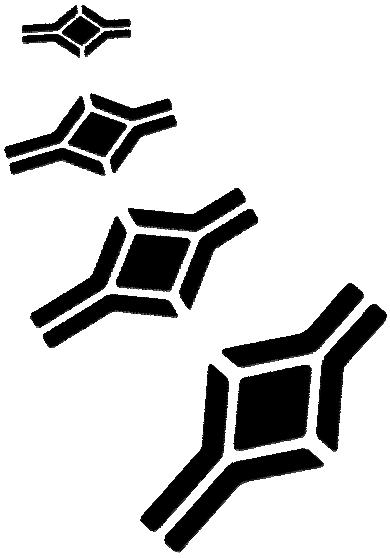

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