Version 27-04/24

User's Manual

Monkey Interleukin-12 (p70) ELISA Kit

REF **DEIA4684**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The monkey IL-12 ELISA Kit is an enzyme-linked immunosorbent assay for quantitative detection of monkey Interleukin-12 in cell culture supernatants, monkey serum, plasma or other body fluids. This ELISA specifically recognizes biologically active heterodimeric p70 IL-12. It does not cross react with the p40 antagonist. **The monkey IL-12 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

General Description

Interleukin-12 (IL-12) is a pleiotropic cytokine, formerly termed cytotoxic lymphocyte maturation factor (CLMF) or natural killer cell stimulatory factor (NKSF), which is produced primarily by stimulated macrophages. It was originally identified as a factor produced by human Epstein-Barr Virus transformed B cell lines. Meanwhile IL-12 has 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 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. 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. 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.

Principles of Testing

1. An anti-monkey IL-12 monoclonal coating antibody is adsorbed onto microwells.
2. Monkey IL-12 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-monkey IL-12 antibody is added and binds to monkey IL-12 captured by the first antibody.
3. Following incubation unbound biotin conjugated anti-monkey IL-12 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-monkey IL-12. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.
4. A coloured product is formed in proportion to the amount of monkey IL-12 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 monkey IL-12 standard dilutions and monkey IL-12 sample concentration determined.

Reagents And Materials Provided

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

2. 1 vial (100 µl) **Biotin-Conjugate anti-monkey-IL-12 monoclonal antibody**
3. 1 vial (150 µl) **Streptavidin-HRP**
4. 2 vials **monkey IL-12 Standard**, lyophilized, 500 U/ml upon reconstitution
5. 1 bottle (50 ml) **Wash Buffer Concentrate 20×** (PBS with 1% Tween 20)
6. 1 vial (5ml) **Assay Buffer Concentrate 20×** (PBS with 1% Tween 20 and 10 % BSA)
7. 1 bottle (12ml) **Sample Diluent (Note: In some cases the Sample Diluent contains insoluble white precipitations which do not interfere with the test performance. Use according to protocol.)**
8. 1 vial (15 ml) Substrate Solution
9. 1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)
10. 1 vial (0.4 ml each) **Blue-Dye, Green-Dye, Red-Dye**
11. 4 adhesive **Plate Covers**
12. **Reagent Labels**

Materials Required But Not Supplied

1. 5 ml and 10 ml graduated pipettes
2. 10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips
3. 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Beakers, flasks, cylinders necessary for preparation of reagents
6. Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
7. Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
8. Glass-distilled or deionized water
9. Statistical calculator with program to perform linear regression analysis.

Storage

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Specimen Collection And Preparation

1. Cell culture supernatants, monkey 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°C to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive monkey IL-12. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C. Avoid repeated freeze-thaw cycles.
2. Aliquots of a serum and cell culture samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the monkey IL-12 level determined after 24 h. There was no significant loss of monkey IL-12 immunoreactivity during storage at -20°C, 4°C and room temperature. Storage at 37°C

gave rise notable loss of monkey IL-12 immunoreactivity.

Reagent Preparation

1. **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 2-25°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

2. **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 2º to 8°C. Please not 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

3. **Preparation of Biotin-Conjugate**

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** with **Assay Buffer** (reagent B) 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

4. **Preparation of monkey IL-12 Standard**

Reconstitute monkey IL-12 **Standard** by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard. Make sure the contents entirely dissolve by gentle swirling.

5. **Preparation of Streptavidin-HRP**

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

Number of Strips	Streptavidin- HRP (ml)	Assay Buffer (ml)
1-6	0.030	6
1-12	0.060	12

6. **Addition of colour-giving reagents**

Blue-Dye, Green-Dye, Red-Dye 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:

a. 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

b. 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 used for the final conjugate dilution. Proceed after addition of **Green-Dye** according to the instruction booklet, preparation of Biotin-conjugate.

5 ml Diluent	20 µl Blue-Dye
12 ml Diluent	48 µl Blue-Dye
12 ml Assay Buffer	120 µl Green-Dye

c. 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 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

Assay Procedure

1. Mix all reagents thoroughly without foaming before use.
2. 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** (murine) to monkey IL-12 from holder and store in foil bag with the desiccant provided at 2-8°C sealed tightly.
3. 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.

4. Add 100 µl of Sample Diluent in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (refer to **Reagent preparation, 4**.) monkey IL-12 Standard, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100µl 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 monkey IL-12 standard dilutions ranging from 250 to 3.9 U/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of monkey IL-12 standard dilutions:

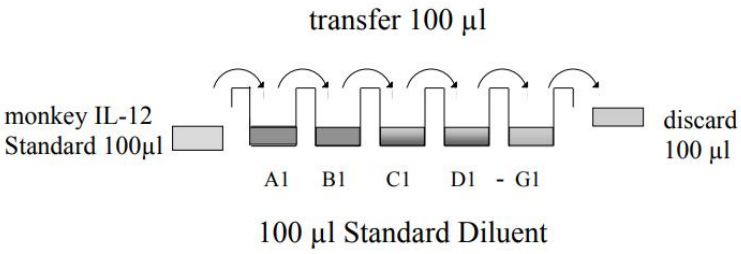


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (250 U/ml)	Standard 1 (250 U/ml)	Sample 1	Sample 1
B	Standard 2 (125 U/ml)	Standard 2 (125 U/ml)	Sample 2	Sample 2
C	Standard 3 (62.5 U/ml)	Standard 3 (62.5 U/ml)	Sample 3	Sample 3
D	Standard 4 (31.3 U/ml)	Standard 4 (31.3 U/ml)	Sample 4	Sample 4
E	Standard 5 (15.6 U/ml)	Standard 5 (15.6 U/ml)	Sample 5	Sample 5
F	Standard 6 (7.8 U/ml)	Standard 6 (7.8 U/ml)	Sample 6	Sample 6
G	Standard 7 (3.9 U/ml)	Standard 7 (3.9 U/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**, in duplicate, to the sample wells.
7. Add 50 µl of each **Sample**, in duplicate, to the designated wells.
8. Prepare **Biotin-Conjugate** (refer to **Reagent Preparation**).
9. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.
10. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 2 hours on a microplate shaker set at 100 rpm.
11. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to point c. of the test protocol. Proceed immediately to the next step.
12. Prepare **Streptavidin-HRP** (refer to**Reagent Preparation**).

13. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.
14. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour on a microplate shaker at 100 rpm.
15. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to point 3. of the test protocol. Proceed immediately to the next step.
16. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
17. Incubate the microwell strips at room temperature (18-25°C) for about 10 minutes. Avoid direct exposure to intense light. **The colour development on the plate should be monitored and the substrate reaction stopped (see point 18. of this protocol) before positive wells are no longer properly recordable.** It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6-0.65 is reached.
18. 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.
19. 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 monkey IL-12 standards.

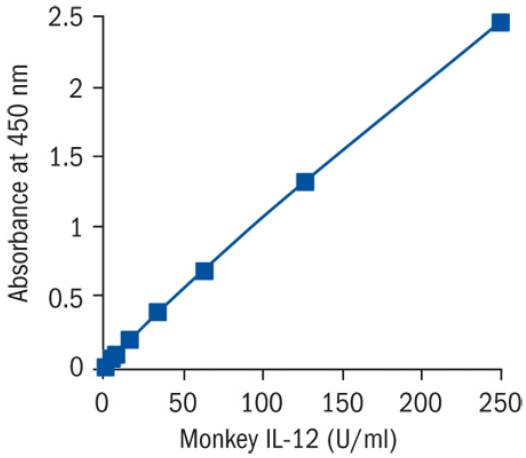
Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Calculation

1. Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the monkey IL-12 concentration on the abscissa. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating monkey IL-12 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 monkey IL-12 concentration. **For samples which 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 dilution factor (x2). Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low monkey IL-12 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual monkey IL-12 level.**

   It is suggested that each testing facility establishes a control sample of known monkey IL-12 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.

Typical Standard Curve



Detection Range

3.9-250 U/ml

Detection Limit

1.6 U/ml

Sensitivity

The limit of detection of monkey IL-12 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 less than 2.64 U/ml (mean of 6 independent assays).

Specificity

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

Linearity

Four serum samples with different levels of monkey IL-12 were assayed at four serial two-fold dilutions with 4 replicates each. The overall mean recovery was 103%.

Recovery

The spiking recovery was evaluated by spiking four levels of monkey IL-12 into different pooled monkey sera. The overall mean recovery was 105%.

Reproducibility

1. Intra-assay

Reproducibility within the assay was evaluated in independent experiments. The overall intra-assay coefficient of variation has been calculated to be <5%.

2. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in independent experiments: The overall inter-assay coefficient of variation has been calculated to be <5%.

Precautions

1. 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.
2. Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
3. Do not mix or substitute reagents with those from other lots or other sources.
4. Do not use kit reagents beyond expiration date on label.
5. Do not expose kit reagents to strong light during storage or incubation.
6. Do not pipette by mouth.
7. Do not eat or smoke in areas where kit reagents or samples are handled.
8. Avoid contact of skin or mucous membranes with kit reagents or specimens.
9. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
10. Avoid contact of substrate solution with oxidizing agents and metal.
11. Avoid splashing or generation of aerosols.
12. 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.
13. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
14. Exposure to acids will inactivate the conjugate.
15. Glass-distilled water or deionized water must be used for reagent preparation.
16. Substrate solution must be at room temperature prior to use.
17. 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.
18. 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.

Limitations

1. Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
2. Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
3. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
4. 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.

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