

BD OptEIA™

Human IL-12 (p40)

ELISA Kit II

Instruction Manual

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Table of Contents

Introduction..... 4

Principle of the Test..... 4

Reagents Provided..... 5

Materials Required but not Provided..... 5

Storage Information..... 6

Warnings and Precautions..... 6

Specimen Collection and Handling..... 7

Reagent Preparation..... 8

Assay Procedure..... 9

Assay Procedure Summary..... 10

Calculation of Results..... 11

Typical Data..... 11

Limitations of the Procedure..... 12

Performance..... 12

 Limit of Detection..... 12

 Recovery..... 12

 Linearity..... 13

 Specificity..... 13

 Precision..... 14

 Intra-assay..... 14

 Inter-assay..... 14

 Standardization..... 14

 Experimental Results..... 14

 Serum..... 14

 Plasma..... 14

 Cell Culture Supernatants..... 15

Troubleshooting..... 15

References..... 15

Plate Templates..... 16

Introduction

Interleukin 12 (IL-12) is a potent regulator of cell-mediated immune responses. Biologically active IL-12 is secreted by activated B lymphocytes and macrophages as a 70 kD heterodimeric glycoprotein comprised of disulfide-bonded 35 kD (p35) and 40 kD (p40) subunits. The IL-12 p40 monomer shares amino acid sequence homology with the IL-6 receptor. It has been reported that activated PBMCs produce a manyfold excess of IL-12 p40 monomer over the bioactive p70 heterodimer. The IL-12 p40 monomer has been reported to inhibit binding of IL-12 p70 to the IL-12 receptor, but with 20× less effectiveness than the IL-12 p70 homodimer.

The BD OptEIA™ ELISA Kit II format was developed for superior accuracy with serum and plasma specimens. The data that demonstrates this enhancement can be located in the Performance “Recovery” and “Linearity” sections.

The BD OptEIA Human IL-12 (p40) ELISA Kit II is for the quantitative determination of human IL-12 (p40) in serum, plasma, and cell culture supernatant.

Principle of the Test

The BD OptEIA test is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for IL-12 (p40) coated on a 96-well plate. Standards and samples are added to the wells, and any IL-12 (p40) present binds to the immobilized antibody. The wells are washed and Streptavidin-horseradish peroxidase conjugate mixed with biotinylated anti-human IL-12 (p40) antibody is added, producing an antibody-antigen-antibody “sandwich”. The wells are again washed and TMB substrate solution is added, which produces a blue color in direct proportion to the amount of IL-12 (p40) present in the initial sample. The Stop Solution changes the color from blue to yellow, and the microwell absorbances are read at 450 nm.

Reagents Provided

| | |
|-------------------------------------|--|
| Antibody Coated Wells: | 2 plates of 96 breakable wells (12 strips \times 8 wells) coated with anti-human IL-12 (p40) monoclonal antibody |
| Detection Antibody: | 30 mL of biotinylated anti-human IL-12 (p40) monoclonal antibody with ProClin®-150 as preservative |
| Standards: | 4 vials lyophilized recombinant human IL-12 (p40) |
| Enzyme Concentrate (250 \times): | 150 μ L of concentrated Streptavidin-horseradish peroxidase conjugate with BSA* and ProClin®-150 as preservative |
| Standard/Sample Diluent: | 30 mL of animal serum* with 0.09% sodium azide as preservative |
| ELISA Diluent: | 12 mL of a buffered protein base with 0.09% sodium azide as preservative |
| Wash Concentrate (20 \times): | 100 mL of 20 \times concentrated detergent solution with ProClin®-150 as preservative |
| TMB One-Step Substrate Reagent: | 30 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution |
| Stop Solution: | 13 mL of 1 M phosphoric acid |
| Plate Sealers: | 4 sheets with adhesive backing |

**Source of all serum proteins is from USDA inspected abattoirs located in the United States*

Materials Required but not Provided

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 50 μ L and 100 μ L volumes
- Adjustable 1 mL, 5 mL, 10 mL, 25 mL pipettes for reagent preparation
- Deionized or distilled water
- Wash bottle or automated microplate washer
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard dilutions
- Laboratory timer
- Absorbent paper

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Storage Information

1. Store kit at 2 - 8°C. Do not use kit after expiration date.
2. Before use, bring all reagents to room temperature (18 - 25°C). Immediately after use, return to proper storage conditions.
3. Lyophilized standards are stable until kit expiration date. After reconstitution, use freshly reconstituted standard within 12 hours (stored at 2 - 8°C).

Warnings and Precautions

1. Reagents that contain preservatives may be toxic if ingested, inhaled, or brought in contact with skin.
2. Avoid contact of skin, eyes, or clothing with Stop Solution or Substrate Reagents.
3. Handle all serum and plasma specimens in accordance with NCCLS guidelines for preventing transmission of blood-borne infections.
4. Standard/Sample Diluent and ELISA Diluent contain less than 0.1% sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. **Warning**

Wash Concentrate (20X) (component 51-9003738) contains 0.002% (w/w), Human IL-12 (p40) Lyophilized Standard (component 51-27426E) contains 0.03% (w/w) and Detection Antibody Biotin Anti-Human IL-12 (p40) (component 51-27422E) contains 0.003% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).

Hazard statements

May cause an allergic skin reaction.

Precautionary statements

Wear protective gloves / eye protection.

Wear protective clothing.

Avoid breathing mist/vapours/spray.

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If skin irritation or rash occurs: Get medical advice/attention.

IF ON SKIN: Wash with plenty of water.

Dispose of contents/container in accordance with local/regional/national/international regulations.

6. Danger

Stop Solution (component 51-2608KC) contains 15.23% phosphoric acid (w/w).

Hazard statements

Causes severe skin burns and eye damage.

Precautionary statements

Wear protective gloves / eye protection.

Wear protective clothing.

IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Continue rinsing.

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Dispose of contents/container in accordance with local/regional/national/international regulations.

Specimen Collection and Handling

Specimens should be clear, non-hemolyzed and non-lipemic. Samples with expected values higher than the top standard, 2000 pg/mL, should be diluted with Standard/Sample Diluent prior to running the assay.

Cell culture supernatants: Remove any particulate material by centrifugation and assay immediately or store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

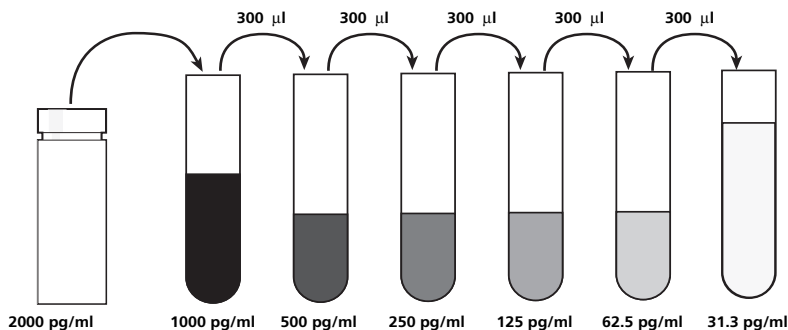
Serum: Use a serum tube (eg, BD Vacutainer® Cat. No. 366430) and allow samples to clot for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum and assay immediately or store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

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Plasma: Collect plasma using citrate, EDTA, or heparin as anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Reagent Preparation

1. Bring all reagents to room temperature ($18 - 25^{\circ}\text{C}$) before use.
2. Standards
 - a. Reconstitute 1 vial lyophilized Standard with required volume (noted on vial label) of Standard/Sample Diluent to prepare a 2000 pg/mL stock standard. Allow the standard to equilibrate for at least 15 minutes before making dilutions. Gently vortex to mix.
 - b. Add 300 μL Standard/Sample Diluent to 6 tubes. Label as 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, and 31.3 pg/mL.
 - c. Perform serial dilutions by adding 300 μL of each standard to the next tube and vortexing between each transfer. The undiluted standard serves as the high standard (2000 pg/mL). The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



3. Working Detector

Note: One-step incubation of Biotin/Avidin reagents. See *Assay Procedure, step 5*.

4. Wash Buffer

Note: If the Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute required quantity of 20× Wash Concentrate with deionized or distilled water, mix. (To prepare 2,000 mL, add 100 mL Wash Concentrate to 1,900 mL water. At least 500 mL solution should be prepared for a full 96-well plate).

5. TMB One-Step Substrate Reagent

No more than 15 minutes prior to use, add required volume of TMB One-Step Substrate Reagent to a clean tube or reservoir. To prevent contamination, pipette out from the tube/reservoir instead of directly from bottle. Avoid prolonged exposure to light or contact with metal, air, or extreme temperature as color may develop.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) prior to use. It is recommended that all standards and samples be run in duplicate. A standard curve is required in each assay run.
2. Remove required quantity of test strips/wells, place in well holder.

Note: Wells are provided in breakable 8-well strips. Strips may be “broken” into individual wells, replaced in well holder, and assayed. Return any unused wells to sealed pouch for 2 - 8°C storage.
3. Pipette 50 µL of ELISA Diluent into each well.
4. Pipette 100 µL of each standard (see *Reagent Preparation, step 2*) and sample into appropriate wells. Gently shake/tap the plate for 5 seconds to mix. Cover wells with Plate Sealer and incubate for 2 hours at room temperature.
5. Prepare Working Detector. Within 15 minutes prior to use, pipette required volume of Detection Antibody into a clean tube or flask. Add in required quantity of Enzyme Concentrate (250×), vortex or mix well. For a full 96-well plate, add 48 µL of Enzyme Concentrate into 12 mL of Detection Antibody.
6. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µL/well prepared Wash Buffer (see *Reagent Preparation, step 4*), followed by decanting/aspirating. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove any residual buffer. Complete removal of liquid is required for proper performance.

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7. Add 100 μ L of prepared Working Detector (see *step 5 above*) to each well. Cover wells with Plate Sealer and incubate for 1 hour at room temperature.
8. Wash wells as in Step 6, but a total of 7 times.

Note: In this final wash step, soak wells in wash buffer for 30 seconds to 1 minute for each wash. Thorough washing at this step is very important.
9. Add 100 μ L of TMB One-Step Substrate Reagent to each well. Incubate plate (without Plate Sealer) for 30 minutes at room temperature in the dark.
10. Add 50 μ L of Stop Solution to each well.
11. Read absorbance at 450 nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570 nm from readings at 450 nm.

Assay Procedure Summary

1. Add 50 μ L ELISA Diluent to each well.
2. Add 100 μ L standard or sample to each well.
Incubate 2 hours at room temperature.
3. Aspirate and wash 5 times.
4. Add 100 μ L prepared Working Detector to each well.
Incubate 1 hour at room temperature.
5. Aspirate and wash/soak 7 times.
6. Add 100 μ L TMB One-Step Substrate Reagent to each well.
Incubate 30 minutes at room temperature.
7. Add 50 μ L Stop Solution to each well.
Read at 450 nm within 30 minutes.
 λ correction 570 nm.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance (ie, plate background) from each.

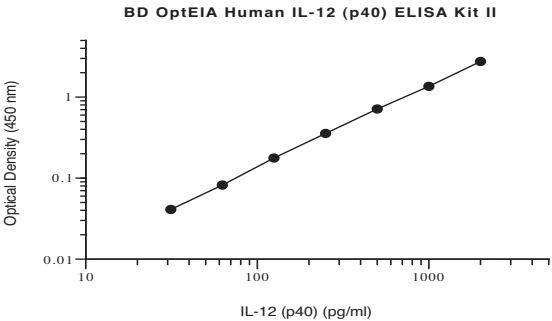
Plot the standard curve on log-log graph paper, with IL-12 (p40) concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points.

To determine the IL-12 (p40) concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the IL-12 (p40) concentration. If samples were diluted, multiply the interpolated IL-12 (p40) concentration by the dilution factor.

Computer-based curve-fitting statistical software may also be employed.

Typical Data

This standard curve is for demonstration only. A standard curve must be run with each assay.



| Concentration (pg/mL) | OD1 | OD2 | Mean | Zero Standard Subtracted |
|-----------------------|-------|-------|-------|--------------------------|
| 0 | 0.014 | 0.016 | 0.015 | 0.000 |
| 31.3 | 0.055 | 0.057 | 0.056 | 0.041 |
| 62.5 | 0.095 | 0.098 | 0.097 | 0.082 |
| 125 | 0.189 | 0.195 | 0.192 | 0.177 |
| 250 | 0.365 | 0.376 | 0.371 | 0.356 |
| 500 | 0.708 | 0.748 | 0.728 | 0.713 |
| 1000 | 1.318 | 1.426 | 1.372 | 1.357 |
| 2000 | 2.757 | 2.775 | 2.766 | 2.751 |

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Limitations of the Procedure

1. Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.
2. This kit is intended for use as an integral unit. Do not mix reagents from different kit lots. Reagents from other manufacturers/other available antibody clones should not be used in this kit.

Performance

Limit of Detection

The minimum detectable dose of IL-12 (p40) was determined to be 3.9 pg/mL. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standard.

Recovery

Three different levels of IL-12 (p40) were spiked into samples of various matrices. Results are compared with the same amounts of IL-12 (p40) spiked into Standard/Sample Diluent, as follows:

| | Spike Concentration (pg/mL) | Average % Recovery | Range |
|-------------------------------|--------------------------------|-----------------------|-----------|
| Serum (n = 6) | 1000 | 70 | 65 - 73 |
| | 500 | 72 | 66 - 73 |
| | 250 | 77 | 73 - 81 |
| Plasma (n = 7) | 1000 | 81 | 76 - 84 |
| | 500 | 80 | 75 - 88 |
| | 250 | 97 | 78 - 103 |
| Cell culture media (n = 3) | 1000 | 96 | 90 - 100 |
| | 500 | 105 | 97 - 109 |
| | 250 | 110 | 100 - 116 |

Linearity

Samples spiked with high concentrations of IL-12 (p40) were serially diluted with Standard Diluent and run in the BD OptEIA Kit. Results are as follows:

| Dilution | | Serum (n = 6) | Plasma (n = 7) | Cell culture media (n = 3) |
|----------|----------------------------|------------------|-------------------|-------------------------------|
| 1:2 | Average% of Expected Range | 119 119 - 127 | 115 111 - 112 | 98 95 - 99 |
| 1:4 | Average% of Expected Range | 130 124 - 136 | 120 115 - 124 | 95 92 - 97 |
| 1:8 | Average% of Expected Range | 134 129 - 139 | 117 106 - 124 | 93 86 - 98 |
| 1:16 | Average% of Expected Range | 135 126 - 142 | 112 100 - 121 | 85 78 - 95 |

Specificity

Cross-Reactivity: The factors listed below were spiked in Standard Diluent at 100 ng/mL to test for any cross reactivity with the BD OptEIA Human IL-12 (p40) ELISA assay. No cross reactivity was identified.

Recombinant Human

sCD23, Eotaxin, SFas, GM-CSF, Gro- α , Gro- β , Gro- γ , I-309, IFN- γ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p70), IL-15, IL-16, IP-10, MCP-1, MCP-2, MCP-3, MIG, MIP-1 α , MIP-1 β , NAP-2, PF-4, SDF-1a, TNF- α , TNF- β

Recombinant Mouse

IFN- γ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), GM-CSF, MCP-1, MIG, MIP-1 α , MIP-1 β , TCA-3, TNF- α

Recombinant Rat

IL-2, IL-10, GM-CSF, MCP-1, RANTES

Precision

Intra-assay

Twenty-four replicates each of three different levels of IL-12 (p40) were tested in one plate. The following results were observed:

| | | | |
|-----------------------------|-------------|-------------|-------------|
| Number of replicates | 24 | 24 | 24 |
| Mean Concentration | 911.4 pg/mL | 446.0 pg/mL | 212.8 pg/mL |
| SD | 45.0 | 29.5 | 15.9 |
| %CV | 4.9 | 6.6 | 7.5 |

Inter-assay

Three different levels of IL-12 (p40) were tested in four different plates. The following results were observed:

| | | | |
|-----------------------------|-------------|-------------|-------------|
| Number of replicates | 32 | 32 | 32 |
| Mean Concentration | 874.7 pg/ml | 459.4 pg/mL | 236.6 pg/mL |
| SD | 47.3 | 19.9 | 12.1 |
| %CV | 5.4 | 4.3 | 5.1 |

Standardization

This immunoassay is calibrated against recombinant human IL-12 (p40).

Experimental Results

Serum

Eighteen serum samples were tested in this assay. The mean value was 66.0 pg/mL, with a range from 28.5 pg/mL to 149.0 pg/mL.

Plasma

Twenty plasma samples were tested in this assay. The mean value was 82.5 pg/mL, with a range from 34.6 to 209.3 pg/mL.

Cell Culture Supernatants

Human peripheral blood mononuclear cells were cultured in RPMI 1640 complete medium with 10% fetal bovine serum at 1×10^6 cells/mL.

The sample was stimulated with hIFN- γ for 2 hours at 37°C, then LPS at 1 μ g/mL and incubated for 24 hours at 37°C.

The result for this sample in the BD OptEIA Human IL-12 (p40) ELISA Kit II was 3902.9 pg/mL.

Troubleshooting

| Problem | Possible Source | Corrective Action |
|---------------------|--|--|
| Poor Precision | <ul style="list-style-type: none"> • Inadequate washing / aspiration of wells • Inadequate mixing of reagents • Imprecise / inaccurate pipetting • Imprecise sealing of plate | <ul style="list-style-type: none"> • Check function of washing system • Ensure adequate mixing • Check / calibrate pipettes • Ensure complete sealing of plate |
| Poor Standard Curve | <ul style="list-style-type: none"> • Improper standard handling / dilution • Incomplete washing / aspiration of wells • Imprecise / inaccurate pipetting | <ul style="list-style-type: none"> • Ensure correct preparation of standards • Check function of washing system • Check / calibrate pipettes |
| Low Signal | <ul style="list-style-type: none"> • Inadequate reagent volumes added to wells • Incorrect incubation times / temperature • Overly high wash / aspiration pressure from automated plate-washer. | <ul style="list-style-type: none"> • Check / calibrate pipettes • Ensure sufficient incubation times / reagents warmed to room temperature • Utilize manual washing |

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Plate Templates

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

Notes

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Notes

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United States

877.232.8995

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866.979.9408

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32.2.400.98.95

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0120.8555.90

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65.6861.0633

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55.11.5185.9995



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