



BD OptEIA™

Human IL-12 (p40)

ELISA Kit II

Instruction Manual

Cat. No. 551116



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## BD OptEIA™ ELISA Kits and Sets available from BD Biosciences – Pharmingen:

### BD OptEIA ELISA Kits

Human	Cat. No.
C3a	550499
C4a	550947
C5a	550500
C5a Kit II	557965
IFN- $\gamma$ Kit II	550612
IL-1 $\beta$	559111
IL-1 $\beta$ Kit II	557966
IL-2 Kit II	550611
IL-4 Kit II	550614
IL-6 Kit II	550799
IL-8 Kit II	550999
IL-10 Kit II	550613
IL-12 p40 Kit II	551116
IL-12 p70	559258
MCP-1	559017
TNF Kit II	550610

### Mouse

IFN- $\gamma$	550582
IL-6	550950
TNF	559732

### Rat

TNF	550734
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### BD OptEIA ELISA Sets

(Capture antibody, Detection antibody, Streptavidin-HRP, and Standard for 5 or 20 ELISA plates).

### Human

Active Caspase-3 (5 plate)	inquire
Cleaved PARP (5 plate)	552592
Eotaxin	555175
GM-CSF	555126
IFN- $\gamma$	555142
IL-1 $\beta$	558848
IL-1 $\beta$ Set II	557953
IL-2	555190
IL-2 sR $\alpha$	559104
IL-3	558979
IL-4	555194
IL-5	555202
IL-6	555220
IL-8	555244
IL-10	555157
IL-12 p40	555171
IL-12 p70	555183
IL-15	559268
IP-10	550926
LT- $\alpha$ (TNF- $\beta$ )	550995
MCP-1	555179

### BD OptEIA ELISA Sets *continued*

Human	
MIG	550998
FAS	555224
sICAM-1	551424
TNFRI	550996
TGF- $\beta$	559119
TNF	555212
TRAIL	550948

### Mouse

GM-CSF	555167
IFN- $\gamma$	555138
IFN- $\gamma$ (AN-18)	551866
IgE	555248
IL-1 $\alpha$	550347
IL-1 $\beta$	559603
IL-2	555148
IL-3	555228
IL-4	555232
IL-5	555236
IL-6	555240
IL-10	555252
IL-12 p40	555165
IL-12 p70	555256
MCP-1	555260
TNF (Mono/Mono)	555268
TNF (Mono/Polys)	558874

### Rat

IFN- $\gamma$	558861
IL-4	555198
IL-6	550319
IL-10	555134
MCP-1	555130
TNF	558870

### Monkey

IFN- $\gamma$	551492
IL-2	551494

### BD ELISA Kits

Canine C-Reactive Protein (CRP)	557826
Rat C-Reactive Protein (CRP)	557825

# Table of Contents

Introduction . . . . .	5
Intended Use . . . . .	5
Principle of the Test . . . . .	5
Reagents Provided . . . . .	6
Materials Required but not Provided . . . . .	6
Storage Information . . . . .	6
Warnings and Precautions . . . . .	7
Specimen Collection and Handling . . . . .	7
Reagent Preparation . . . . .	8
Assay Procedure . . . . .	9
Assay Procedure Summary . . . . .	10
Calculation of Results . . . . .	10
Typical Data . . . . .	11
Limitations of the Procedure . . . . .	11
Performance Characteristics . . . . .	12
Sensitivity . . . . .	12
Recovery . . . . .	12
Linearity . . . . .	12
Specificity . . . . .	13
Precision . . . . .	13
Intra-assay . . . . .	13
Inter-assay . . . . .	13
Standardization . . . . .	13
Expected Values . . . . .	14
Serum . . . . .	14
Plasma . . . . .	14
Cell Culture Supernatant . . . . .	14
Troubleshooting . . . . .	14
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 manifold 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 Characteristics “Recovery” and “Linearity” sections.

## Intended Use

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 × 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 0.15% ProClin™-150 as preservative
Standards:	4 vials lyophilized recombinant human IL-12 (p40)
Enzyme Concentrate (250×):	150 µl of concentrated Streptavidin-horseradish peroxidase conjugate with BSA* and ProClin™-300 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×):	100 ml of 20× 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 1M 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 µl and 100 µl 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

## 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. TMB One-Step Substrate Reagent is toxic if inhaled or swallowed. Avoid contact with skin. Keep container tightly closed. In case of an accident or if you feel unwell, seek medical advice immediately.
5. 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.
6. The Wash Concentrate contains 16% Sodium Chloride and is an irritant.
  - R36/37/38 Irritating to eyes, respiratory system, and skin.
  - S7 Keep container tightly closed.
  - S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
  - S36/37 Wear suitable protective clothing and gloves.
  - S60 This material and its container must be disposed of as hazardous waste.
7. The Stop Solution contains 11.5% Phosphoric Acid and is a corrosive solution.
  - R34 Causes burns.
  - R36/38 Irritating to eyes and skin.
  - S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
  - S37 Wear suitable gloves.
  - S45 In case of accident or if you feel unwell, seek medical advice immediately.
  - S60 This material and its container must be disposed of as hazardous waste.

## 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.

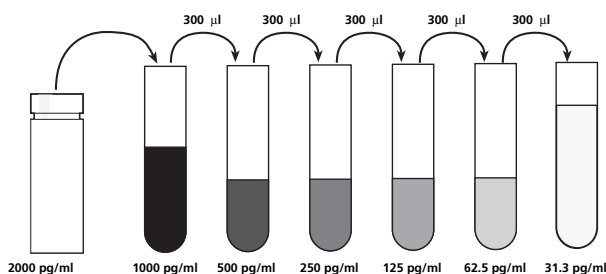
**Cell culture supernatants:** Remove any particulate material by centrifugation and assay immediately or store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**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  $1000 \times g$ . Remove serum and assay immediately or store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Plasma:** Collect plasma using citrate, EDTA, or heparin as anticoagulant. Centrifuge for 10 minutes at  $1000 \times g$  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°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  $\mu$ l 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  $\mu$ l of each standard to the next tube and vortexing between each transfer (see *figure* below). 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/Streptavidin 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 $\times$  Wash Concentrate with deionized or distilled water, mix. (To prepare 2.0 L, add 100 ml Wash Concentrate to 1900 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.
7. Add 100 µl 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 µl 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 µl 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  $\mu\text{l}$  ELISA Diluent to each well.
2. Add 100  $\mu\text{l}$  standard or sample to each well.  
Incubate 2 hours at room temperature.
3. Aspirate and wash 5 times.
4. Add 100  $\mu\text{l}$  **prepared** Working Detector to each well.  
Incubate 1 hour at room temperature.
5. Aspirate and wash/soak 7 times.
6. Add 100  $\mu\text{l}$  TMB One-Step Substrate Reagent to each well.  
Incubate 30 minutes at room temperature.
7. Add 50  $\mu\text{l}$  Stop Solution to each well.  
Read at 450 nm within 30 minutes.  
 $\lambda$  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.

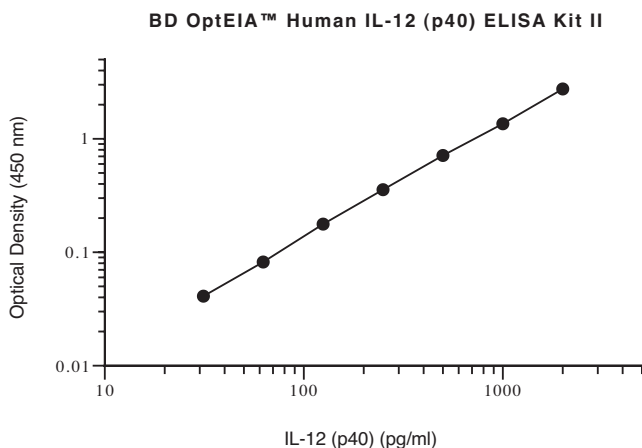
Plot the standard curve on log-log graph paper, with IL-12 (p40) concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points.

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

## 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 Characteristics

## Sensitivity

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:

	<b>Spike Concentration (pg/ml)</b>	<b>Average % Recovery</b>	<b>Range</b>
<b>Serum (n = 6)</b>	1000	70	65 - 73
	500	72	66 - 73
	250	77	73 - 81
<b>Plasma (n = 7)</b>	1000	81	76 - 84
	500	80	75 - 88
	250	87	78 - 103
<b>Cell culture media (n = 3)</b>	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/Sample Diluent and run in the BD OptEIA™ Kit.

Results are as follows:

<b>Dilution</b>		<b>Serum (n = 6)</b>	<b>Plasma (n = 7)</b>	<b>Cell culture media (n = 3)</b>
1:2	Average % of Expected	119	115	98
	Range	119 - 127	111 - 122	95 - 99
1:4	Average % of Expected	130	120	95
	Range	124 - 136	115 - 124	92 - 97
1:8	Average % of Expected	134	117	93
	Range	129 - 139	106 - 124	86 - 98
1:16	Average % of Expected	135	112	85
	Range	126 - 142	100 - 121	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- $\alpha$ , Gro- $\beta$ , Gro- $\gamma$ , I-309, IFN- $\gamma$ , 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 $\alpha$ , MIP-1 $\beta$ , NAP-2, PF-4, SDF-1a, TNF- $\alpha$ , TNF- $\beta$

### Recombinant Mouse

IFN- $\gamma$ , 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 $\alpha$ , MIP-1 $\beta$ , TCA-3, TNF- $\alpha$

### 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 baculovirus-expressed recombinant human IL-12 (p40) produced at BD Biosciences – Pharmingen.

## Expected Values

**Serum:** Eighteen apparently healthy normal donors' 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 apparently healthy normal donors' 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 supernatant:

Human peripheral blood mononuclear cells from an apparently healthy, normal donor were cultured in RPMI 1640 complete medium with 10% fetal bovine serum at  $1 \times 10^6$  cells/ml.

The sample was stimulated with hIFN- $\gamma$  for 2 hours at 37°C, then LPS at 1  $\mu$ 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"><li>• Inadequate washing / aspiration of wells</li><li>• Inadequate mixing of reagents</li><li>• Imprecise / inaccurate pipetting</li><li>• Imprecise sealing of plate</li></ul>	<ul style="list-style-type: none"><li>• Check function of washing system</li><li>• Ensure adequate mixing</li><li>• Check / calibrate pipettes</li><li>• Ensure complete sealing of plate</li></ul>
Poor Standard Curve	<ul style="list-style-type: none"><li>• Improper standard handling / dilution</li><li>• Incomplete washing / aspiration of wells</li><li>• Imprecise / inaccurate pipetting</li></ul>	<ul style="list-style-type: none"><li>• Ensure correct preparation of standards</li><li>• Check function of washing system</li><li>• Check / calibrate pipettes</li></ul>
Low Signal	<ul style="list-style-type: none"><li>• Inadequate reagent volumes added to wells</li><li>• Incorrect incubation times / temperature</li><li>• Overly high wash / aspiration pressure from automated plate-washer.</li></ul>	<ul style="list-style-type: none"><li>• Check / calibrate pipettes</li><li>• Ensure sufficient incubation times / reagents warmed to room temperature</li><li>• Utilize manual washing</li></ul>

## References

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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												







**United States**

877.232.8995

**Canada**

888.259.0187

**Europe**

32.53.720.211

**Japan**

0120.8555.90

**Asia/Pacific**

65.6861.0633

**Latin America/Caribbean**

55.11.5185.9995



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