Calculation of Results
Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
Plot the standard curve on log-log graph paper, with IFN-γ concentration on the x-axis and absorbance on the y-axis. Draw the best fit curve through the standard points.
To determine the IFN-γ concentration of the unknowns, find the unknown’s 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 IFN-γ concentration. If samples were diluted, multiply the IFN-γ concentration by the dilution factor.
Computer data reduction may also be employed, utilizing log-log regression analysis.

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

Standardization
This immunoassay is calibrated against recombinant human IFN-γ.
The NIBSC/WHO First British Standard 82/587 (human IFN-γ prepared by induction of phytophaemagglutinin) was evaluated in this set. The conversion factor for NIBSC material is as follows:
NIBSC equivalent value (IU/mL) = 0.010 x BD OptEIA™ Human IFN-γ (pg/mL).
The NIAID Standard #Gxg01-902-535 (recombinant human IFN-γ expressed in E. coli) was evaluated in this set. The conversion factor for NIAID material is as follows:
NIAID equivalent value (IU/mL) = 0.021 x BD OptEIA™ IFN-γ value (pg/mL).

Assay Optimization
1. BD OptEIA™ Sets allow flexible assay design to fit individual laboratory needs. To design an immunoassay with different sensitivity and dynamic range, the following parameters can be varied: Capture, Detection Antibody titers, Incubation time, Incubation temperature, Assay Diluent formulation, Buffer pH, ionic strength, protein concentration, Type of substrate, Washing technique (i.e., number of wash repetitions and soak times)
2. “Typical Standard Curve” and 20-plate yield were obtained in the BD Biosciences Pharmingen laboratory, using the recommended procedure and manual plate washing.

Troubleshooting

Poor Precision
Possible Source
• Inadequate washing/ aspiration of wells
• Inadequate mixing of reagents
• Improper/ inaccurate pipetting
• Incomplete sealing of plate
Corrective Action
• Check function of washing system
• Ensure adequate mixing
• Check/ calibrate pipettes
• Ensure complete seal on plate

Poor Standard Curve
Possible Source
• Improper standard handling/ dilution standard
• Incomplete washing/ aspiration of wells
• Improper/ inaccurate pipetting
• Improper buffer/ detergent used
Corrective Action
• Ensure correct preparation, storage of
• Check function of washing system
• Check/ calibrate pipettes
• Check buffer/ detergent preparation, pH

Low Absorbances
Possible Source
• Inadequate reagent volumes added to wells
• Incorrect incubation times/ temperature
• Incorrect antibody titration
• Improper buffer/ detergent used
• Excessively high wash/ aspiration pressure from automated plate-washer
Corrective Action
• Check/ calibrate pipettes
• Ensure sufficient incubation times/temperatures warned to RT
• Check Capture Ab and Working
• Check buffer/ detergent preparation, pH
• Utilize manual washing

Limitations of the Procedure
• Samples that generate absorbance values higher than the standard curve should be diluted with Standard Diluent and re-assayed.
• Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.
• BD OptEIA™ Sets are intended for use as an integral unit. Do not mix reagents from different Set batches. Reagents from other manufacturers are not recommended for use in this Set.

Specificity
Cross Reactivity: The following factors were tested in the BD OptEIA™ assay at 10 ng/mL and no cross-reactivity was identified.
Recombinant Human
IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-13, IL-15, G-CSF, GM-CSF, CD23, Lymphotactin, MIP-1α, MIP-1β, MCP-1, MCP-2, NT-3, PDGF-AA, SCF, TNF, LT-α(TNF-β) VEGF
Recombinant Mouse
IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p70), IL-15, IFN-γ, GM-CSF, MCP-1, TCA3, TNF
Recombinant Rat
IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, TNF
Other
Viral IL-10 (1 ng/ml) Rabbit TNF

Interfering Substances: The following substances at levels greater than or equal to 2 mg/ml were added to Assay Diluent spiked with 200 pg/ml IFN-γ. No effect on assay results was observed. Bilirubin, human γ-globulin, human hemoglobin, human serum albumin, human transferrin, Triglycerides, Heparin (300 units/ml), Sodium Citrate, EDTA.

BD OptEIA™ Human IFN-γ ELISA Set

Materials Provided
The OptEIA™ Set for human interferon-gamma (IFN-γ), contains the components necessary to develop enzyme-linked immunosorbent assays (ELISA) for natural or recombinant human IFN-γ in serum, plasma, and cell culture supernatants. Sufficient materials are provided to yield approximately 20 plates of 96-wells if the recommended storage, materials, buffer preparation, and assay procedure are followed as specified in this package.

Capture Antibody
Anti-Human IFN-γ monoclonal antibody

Detection Antibody
Biotinylated Anti-Human IFN-γ monoclonal antibody

Enzyme Reagent
Streptavidin-horseradish peroxidase conjugate (SAv-HRP)

Standards
Recombinant human IFN-γ, lyophilized

Instruction / Analysis Certificate
(lot-specific)

United States
877.232.8995
Canada
866.979.4908
Europe
32.2.400.98.95
Japan
0120.8555.90
Asia/Pacific
65.6861.0633
Latin America/Caribbean
55.11.5185.9995

2613K1_555142 Rev 6
Recommended Buffers, Solutions

Note: Do not use sodium azide in these preparations. Sodium azide inactivates the horseradish peroxidase enzyme.

The BD OptEIA® Reagent Set B (Cat. No. 550534) containing Coating Buffer, Assay Diluent, Substrate Reagents A and B, Stop Solution and 2X Wash Buffer Concentrate is recommended.

1. **Coating Buffer** - 0.1 M Sodium Carbonate, pH 9.5
    - 7.13 g NaHCO₃, 1.59 g Na₂CO₃; q.s. to 1.0 L; pH 9.5 with 10N NaOH.

2. **Assay Diluent** - PBS* with pH 7.0. The BD Pharmingen™ Assay Diluent (Cat. No. 552513) is recommended.

3. Lyophilized standards are stable until expiration date. See below for Storage/Handling of reconstituted standard.

4. **Wash Buffer** - PBS* with 0.05% Tween-20. Freshly prepare or use within 3 days of preparation, stored at 2-8°C.

5. **Substrate Solution** - Tetramethylbenzidine (TMB) and Hydrogen Peroxide. The BD Pharmingen™ TMB Substrate Reagent Set (Cat. No. 555214) is recommended.

6. **Stop Solution** - 1 M H₂PO₄ or 2 N H₂SO₄

Additional Materials Required
1. 96-well Nunc-Immuno™ polystyrene Maxisorp ELISA flat bottom plates (ThermoFisher Scientific Cat. No. 442404) are recommended.
2. Microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes
4. Graduated cylinder, one liter
5. Deionized or distilled water
6. Wash bottle or automated washer
7. Log-log graph paper or automated data reduction
8. Tubes to prepare standard dilutions
9. Laboratory timer
10. Plate sealers or parafilm

Storage Information
1. Store unopened reagents at 2-8°C. Do not use reagents after expiration date, or if turbidity is evident.
2. Before use, bring all reagents to room temperature (18-25°C).
3. Immediately after use, return to proper storage conditions.
4. Lyophilized standards are stable until expiration date. See below for reconstituted standard storage information.

Specimen Collection and Handling
Specimens should be clear, non-hemolyzed and non-lipemic.

Cell culture supernatants: Remove any particulate material by centrifugation and assay immediately or store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube 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 ≤-20°C. Avoid repeated freeze-thaw cycles.

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 ≤-20°C. Avoid repeated freeze-thaw cycles.

Recommended Assay Procedure

1. 100 µL per well of Capture Antibody diluted in Coating Buffer. For recommended antibody coating dilution, see lot-specific Instruction/Analysis Certificate. See “Standards Preparation and Handling”.
2. Pipette 100 µL of each standard, sample, and control into appropriate wells. Seal plate and incubate for 1 hour at RT.
3. Aspirate wash and add 50 µL Stop Solution to each well. Read absorbance at 450 nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract absorbance at 570 nm from absorbance 450 nm.

Wash Buffer Concentrate is a registered trademark of Rohm and Haas Company.

For information about other BD OptEIA products, visit www.bdbiosciences.com/support/resources