**CALCULATION OF RESULTS**

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the feline IFN-γ concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**TYPICAL DATA**

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed.

![Graph showing typical data](Image)

**SPECIFICITY**

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

**Recombinants:**
- cotton rat IFN-γ
- human IFN-γ
- mouse IFN-γ
- porcine IFN-γ
- primate IFN-γ
- rat IFN-γ

A sample containing 3.1 ng/mL of recombinant canine IFN-γ reads as 594 pg/mL (19% cross-reactivity).

**TECHNICAL HINTS & LIMITATIONS**

- We recommend the use of R&D Systems® Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare the Block Buffer for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent and Block Buffer is crucial for the optimum performance of the DuoSet® ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute or dilute the Streptavidin-HRP A.
- It is important that the Reagent Diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispersed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

**TROUBLESHOOTING**

**Note:** For more detailed troubleshooting, please visit: [www.RnDSystems.com/ELISADevelopment](http://www.RnDSystems.com/ELISADevelopment)

**Poor Standard Curve**
- Impure BSA used for Reagent Diluent and Block Buffer preparation.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

**Poor Precision**
- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

**Low or No color Development**
- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent and Block Buffer preparation.

**INTENDED USE**

For the development of sandwich ELISAs to measure natural and recombinant feline Interferon gamma (IFN-γ). The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on at least fifteen 96 well plates, provided the following conditions are met:
- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product. Refer to the Certificate of Analysis for component concentrations as they may vary. For research use only. Not for use in diagnostic procedures.
**Reagent Preparation**

Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately, unless otherwise noted.

**Streptavidin-HRP A:** 1.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

**Goat Anti-Feline IFN-γ Capture Antibody:** Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

**Biotinylated Goat Anti-Feline IFN-γ Detection Antibody:** Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent with 2% heat inactivated normal goat serum (NGS) to the working concentration indicated on the C of A. Prepare at least 15 minutes prior to use.

**Recombinant Feline IFN-γ Standard:** Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 μL of high standard per plate assayed at the concentration indicated on the C of A.

** Calibration**

This DuoSet® is calibrated against a highly purified E. coli-expressed recombinant feline IFN-γ produced at R&D Systems®.