# VeriKine™ Human Interferon Beta ELISA Kit
# Certificate of Analysis & Protocol

Assay Range: 50 - 4000 pg/ml
Compatibility: Tissue Culture Media
Assay Length: 3 hr 15 min

**Catalog No:** 41410-2
**Lot No: 7723**
**Expiration: January 31, 2026**
Store all components at 2-8ºC

| Kit Components | Part No. | Lot No. | Quantity |
|---|---|---|---|
| Plate(s) | SMP055 | K7477 | 5 |
| Plate Sealers | N/A | N/A | 20 |
| Wash Solution Concentrate | SMP057-250 | K7496 | 250 ml |
| Human IFN-Beta Standard, 100,000 pg/ml | SMP146-2 | K7569 | 1 vial |
| Sample Diluent | SMP067-150 | K7570 | 150 ml |
| Antibody Concentrate | SMP062-2 | K7571 | 1 vial |
| HRP Conjugate Concentrate | SMP056-900 | K7572 | 1 vial |
| Concentrate Diluent | SMP024-150 | K7390 | 150 ml |
| TMB Substrate Solution | KET-60 | 220103D02 | 60 ml |
| Stop Solution | SCY-60 | 78665 | 60 ml |

## Product Performance Specifications

| | |
|---|---|
| Intra-Assay CV | ≤ 8% |
| Inter-Assay CV | ≤ 8% |

## Authorization

**Released by:** _____

**Date:** September 26, 2024

**NOTE:** The methods associated with the collection, storage, and testing of environmental samples have all been reported to affect ELISA results. For example, we have found that protein-free media provides poor results.

**PIPETTING TIPS:** Due to the inherent nature of Human IFN-Beta protein to adhere to plastic surfaces, proper pipetting technique is required to accurately prepare a standard curve and quantitate samples.

**Aspirating:** To avoid protein sticking to outside walls of the pipette tip, ensure it is not immersed in the standard vial when aspirating.

**Dispensing and Diluting:** Proper mixing technique entails pipetting up and down gently 10 times for predilution and S7 dilution; 5 times for subsequent serial dilutions. Thorough, but gentle, pipetting is required to recover all material attached to the inside of the tip. Avoid excessive force or foaming to prevent denaturing.

**CAUTION:** Sample Diluent, Wash Solution Concentrate and Concentrate Diluent contain 0.1% Kathon CG/ICP as a preservative and should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

## PREPARATION OF REAGENTS

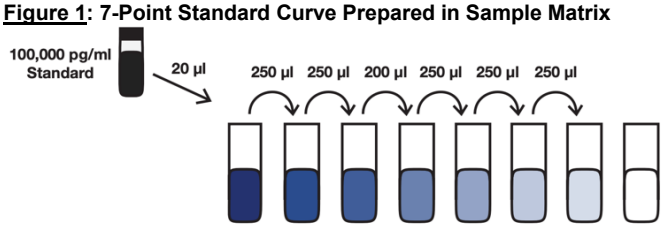
**Wash Buffer:** Wash Solution Concentrate may contain crystals; place in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 Wash Buffer (e.g. Add 50 ml Wash Solution Concentrate to 450 ml distilled or deionized water). Mix thoroughly before use. Store at RT (22-25ºC).

**Human IFN-Beta Solution:** Using the Human IFN-Beta Standard, construct a standard curve in the same matrix as the test samples. If the sample matrix is not available, the Sample Diluent may be used to prepare the sample curve. In certain situations, test samples may contain substances that can interfere with assay results.

**Human IFN-Beta Standard Curve Preparation:**

**a.** Label seven polypropylene tubes (S1 – S7).
**b.** Add indicated volume of Sample Matrix or Sample Diluent to each tube as indicated in Figure 1.
**c.** Using polypropylene tips, add 20 µl of Human IFN-Beta Standard to S7 and mix gently.
**d.** Remove indicated amount from S7 and add to S6. Repeat to complete series to S1. Set on ice (2-8ºC) until use in step 1.

**Figure 1: 7-Point Standard Curve Prepared in Sample Matrix**




| Label | S7 | S6 | S5 | S4 | S3 | S2 | S1 | Blank |
|---|---|---|---|---|---|---|---|---|
| Sample Matrix (µl) | 480 | 250 | 250 | 300 | 250 | 250 | 250 | 250 |
| IFN-Beta (pg/ml) | 4000 | 2000 | 1000 | 400 | 200 | 100 | 50 | 0 |

**Sample Preparation:** Thaw frozen sample tubes to Room Temperature (RT) (22-25ºC) in either tap water or between the fingertips. If samples require dilution, prepare using Sample Diluent. Set on ice (2-8ºC) until use in step 1. Measurements in duplicate are recommended.

**Antibody Solution:** 15 minutes prior to use in step 2, dilute Antibody Concentrate in volume of Concentrate Diluent shown below. Keep at RT (22-25ºC).

| Micro-plate Strips Used | 2 | 4 | 6 | 8 | 10 | 12 |
|---|---|---|---|---|---|---|
| Antibody Concentrate (µl) | 5 | 10 | 15 | 20 | 25 | 30 |
| Concentrate Diluent (ml) | 2.0 | 4.0 | 6.0 | 8.0 | 10.0 | 12.0 |

**HRP Solution:** 15 minutes prior to use in step 3, dilute HRP Conjugate Concentrate in volume of Concentrate Diluent shown below. Keep at RT (22-25ºC).

| Micro-plate Strips Used | 2 | 4 | 6 | 8 | 10 | 12 |
|---|---|---|---|---|---|---|
| HRP Conjugate Concentrate (µl) | 10 | 20 | 30 | 40 | 50 | 60 |
| Concentrate Diluent (ml) | 2.0 | 4.0 | 6.0 | 8.0 | 10.0 | 12.0 |

## ASSAY PROCEDURE

| Bring to RT (22-25ºC) | Keep at 2-8ºC |
|---|---|
| Plate/Sealers | Human IFN-Beta Standard |
| Sample Diluent | Antibody Concentrate |
| Concentrate Diluent | HRP Conjugate Concentrate |
| Stop Solution | |
| Wash Solution Concentrate | |
| TMB Substrate Solution (Bring to RT in step 3) | |
| Matrices/Samples | |

- **Incubations:** Use plate sealers to cover the plate when directed. All incubations should be conducted in a closed chamber at 22-25ºC or at RT, keeping the plate away from drafts.
- **Plate Washing**: All wells should be filled with a minimum of 250 µl of Wash Solution. Remove plate contents by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry.

**1.** Determine the number of microplate strips required. We recommend running both the standard and samples at least in duplicate. Remove extra microtiter strips from the frame, seal in the foil bag provided and store at 2-8ºC. Unused strips can be used in later assays.

**Total well volume = 100 ul (Step A + Step B)**
Step A: Add **50 µl** of Sample Diluent to every well
Step B: Add **50 µl** of **Standard**, **Test Samples** or **Blank** (Sample Diluent or appropriate dilution matrix) to each designated well.

Cover with Plate Sealer and incubate at RT for 1 hour.

After 1 hour, empty plate contents and wash wells three times.

**2.** Add **100 µl** of diluted **Antibody Solution** to each well.

Cover with Plate Sealer and incubate at RT for 1 hour.

After 1 hour, empty plate contents and wash wells three times.

**3.** Add **100 µl** of **HRP Solution** to each well.

Cover with Plate Sealer and incubate at RT for 1 hour. Warm **TMB Substrate Solution** to RT.

After 1 hour, empty plate contents and wash wells three times.

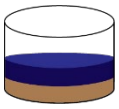
**4.** Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 15 minutes. Do not use a Plate Sealer during the incubation.

**5.** After 15 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

**6.** Using a microplate reader, determine the absorbance at 450 nm within 5 minutes after the addition of Stop Solution.

## HUMAN IFN-BETA ELISA (41410)
## ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 3 hr 15 min
**Note:** All incubations are at Room Temperature (RT) (22-25ºC)*



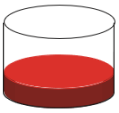
Add **50 µl** Sample Diluent
Add **50 µl** Standard, Sample, or Blank

*Incubate* **1 hr** *at RT**

*Aspirate and Wash* **3x**

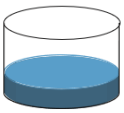
Add **100 µl** diluted Antibody Solution
*Incubate* **1 hr** *at RT**

*Aspirate and Wash* **3x**

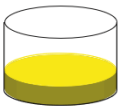


Add **100 µl** diluted HRP Solution
*Incubate* **1 hr** *at RT**

*Aspirate and Wash* **3x**



Add **100 µl** TMB Substrate
*Incubate* **15 min** *in the dark at RT**
*Do not seal, shake, or wash.*

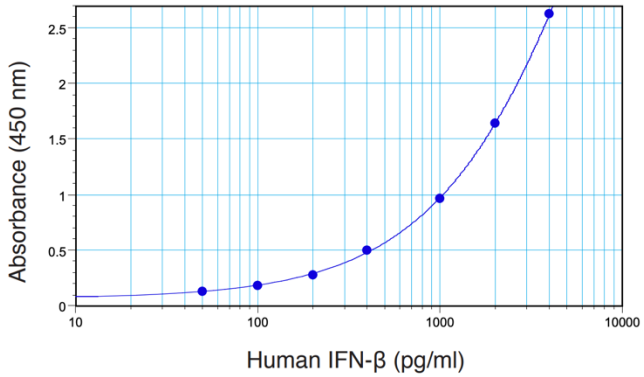


Add **100 µl** Stop Solution
Read plate within 5 min (450 nm)

## CALCULATION OF RESULTS

By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Blank ODs may be subtracted from the standards and sample ODs to eliminate background. An approximate conversion factor of about 3 – 10 pg/unit of Human IFN-Beta, mammalian, is applicable for Human IFN-Beta.

**Figure 2: Typical Standard Curve**




Visit PBL's website (https://pblassaysci.com/documentation) for additional information including technical data sheet

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