

**Catalog No:** 41115-1

**Lot No:** 7781

**Expiration:** October 31, 2026

Store all components at 2-8°C

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP217	K7736	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60	K7693	2 x 50 ml
Human IFN-Alpha Standard, 10,000 pg/ml	SMP049-400	K7740	1 vial
Sample Buffer	SMP220-8	K7745	8 ml
Standard Diluent	SMP218-60	K7741	55 ml
Antibody Concentrate	SMP219-1	K7742	1 vial
Antibody Diluent	SMP304-15	K7758	15 ml
HRP Conjugate Concentrate	SMP056-240	K7744	1 vial
HRP Diluent	ASDHRP-15	974379	15 ml
TMB Substrate Solution	KET-15	241003D02	15 ml
Stop Solution	SCY-15	80958	15 ml

## Authorization

Released by: \_\_\_\_\_

Date: April 29, 2025

## INTRODUCTION

PBL's Human IFN-Alpha All Subtype ELISA quantifies all human IFN-Alpha subtypes and is suitable for use with human sera, plasma, and tissue/cell culture media samples. The standard curve is generated using a single human IFN-Alpha subtype protein standard.

**Note:** Use shaker at 550 rpm speed for optimal assay results.

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

**CAUTION:** Wash Solution Concentrate, Standard Diluent, Sample Buffer, and Antibody 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).

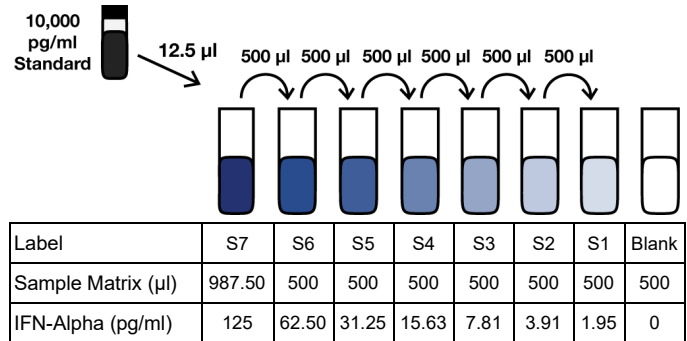
## PREPARATION OF REAGENTS

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

### Human IFN-Alpha Standard Curve Preparation:

- Label seven polypropylene tubes (S1 – S7).
- Add indicated volume of Sample Matrix or Standard Diluent to each tube as indicated in [Figure 1](#).
- Using polypropylene tips, add indicated volume of Human IFN-Alpha Standard to S7 and mix gently. *Change tips between each dilution.*
- Remove indicated amount from S7 and add to S6. Repeat to complete series to S1. Set aside on ice (2-8°C) until use in step 1.

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



**Sample Preparation:** Prepare test samples as required. Measurements in duplicate are recommended. Refrigerate until use in step 1.

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

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (µl)	20	40	60	80	100	120
Antibody 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 the volume of HRP Diluent as shown below. Keep at RT (22-25°C).

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

## ASSAY PROCEDURE

	Bring to RT (22-25°C)	Keep at 2-8°C
Day 1	Plate Sealers	All other components
Day 2	Wash Buffer	All other components
	TMB Substrate Solution (During Step 3)	
	Stop Solution (During Step 3)	

- **Incubations:** All incubations should be conducted in a closed chamber at RT, keeping the plate away from drafts. (**Note:** The overnight incubation is at 4°C and does not require shaking.)
- **Plate Washing:** All wells should be filled with a minimum of 300 µl of Wash Buffer. Remove plate contents by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry.

### DAY 1

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 2-8°C. Unused strips can be used in later assays.

**Total well volume = 100 µl (Step A + Step B)**

**Step A:** Add 50 µl of **Sample Buffer** to every well.

**Step B:** Add 50 µl of **Standard, Test Samples** or **Blanks** (Standard Diluent or appropriate dilution matrix) to each designated well.

Cover with Plate Sealer and shake at 550 rpm at RT for 30 seconds. Transfer the plate to 4°C and incubate for 18-20 hours without shaking.

### DAY 2

After 18-20 hours, empty plate contents and wash wells one time.

2. Add 100 µl of diluted **Antibody Solution** to each well. Cover with Plate Sealer and shake plate at 550 rpm at RT for 1 hour.

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

3. Add 100 µl of diluted **HRP Solution** to each well. Cover with Plate Sealer and shake plate at 550 rpm at RT for 1 hour. Warm **TMB Substrate Solution** and **Stop Solution** to RT.

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

4. Add 100 µl of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 30 minutes. Do not use a Plate Sealer and **DO NOT SHAKE** during the incubation.

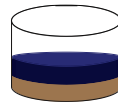
5. After 30 minutes, **DO NOT EMPTY THE WELLS AND DO NOT WASH**. Add 100 µl of **Stop Solution** to each well.

6. Using a microplate reader, determine the absorbance at 450 nm within 2 minutes after the addition of Stop Solution.

## HUMAN IFN-ALPHA ELISA (41115) ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 22 hr 30 min

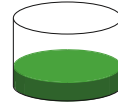
### DAY 1



1. Add 50 µl Sample Buffer
2. Add 50 µl Standard, Sample or Blank  
*Incubate 30 sec (shake at 550 rpm) at RT\**  
*Transfer to 4°C and incubate 18-20 hr*

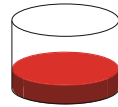
### DAY 2

*Aspirate and Wash 1x*



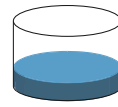
- Add 100 µl diluted Antibody Solution  
*Incubate 1 hr (shake at 550 rpm) at RT\**

*Aspirate and Wash 3x*

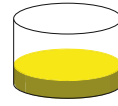


- Add 100 µl diluted HRP Solution  
*Incubate 1 hr (shake at 550 rpm) at RT\**

*Aspirate and Wash 4x*



- Add 100 µl TMB Substrate  
*Incubate 30 min in the dark at RT\**  
Do not seal, shake or wash.



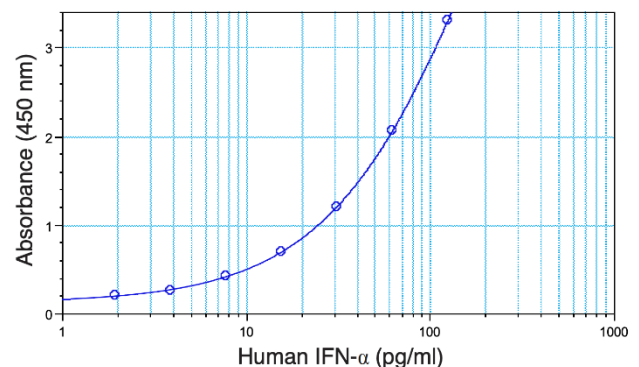
- Add 100 µl Stop Solution  
Read plate within 2 min (450 nm)

**Note:** All incubations are at Room Temperature (RT) (22-25°C)\*

## 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. Use the conversion factor of 3-5 pg/unit to approximate titers in units/ml. Blank ODs may be subtracted from the standards and sample ODs to eliminate background.

**Figure 2: Typical Standard Curve**



Sold under license from Pestka Biomedical Laboratories, Inc. d/b/a PBL Assay Science. For research use only. Not for diagnostic or clinical use in, or administration to, humans. Not for resale in original or any modified form, including inclusion in a kit, for any purpose. Not for use in the preparation of any commercial product. © 2025 Pestka Biomedical Laboratories, Inc. All rights reserved. **Rev. 02**