



VeriKine-HS™ Human IL-15 ELISA Kit

Certificate of Analysis & Protocol

Assay Range: 0.51 – 32.8 pg/ml
 Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
 Assay Length: 4 hr

Catalog No: 41702-1

Lot No: 7612

Expiration: March 31, 2024

Store all components at 2-8°C

| Kit Components | Part No. | Lot No. | Quantity |
|---------------------------------|------------|-----------|-----------|
| Plate(s) | SMP350 | K7189 | 1 |
| Plate Sealers | N/A | N/A | 4 |
| Wash Solution Concentrate | SMP022-60 | K6927 | 2 x 50 ml |
| Human IL-15 Standard, 410 pg/ml | SMP351-1 | K7192 | 1 vial |
| Assay Buffer | SMP352-8 | K7193 | 8 ml |
| Dilution Buffer | SMP021-60 | K7038 | 55 ml |
| Antibody Concentrate | SMP354-1 | K7194 | 1 vial |
| HRP Conjugate Concentrate | SMP056-240 | K7195 | 1 vial |
| Concentrate Diluent | SMP024-15 | K7046 | 15 ml |
| HRP Diluent | ASDHRP-15 | 648946 | 15 ml |
| TMB Substrate Solution | KET-15 | 220103D03 | 15 ml |
| Stop Solution | SCY-15 | 67088 | 15 ml |

Authorization

Released by: _____

Date: March 21, 2023

INTRODUCTION

PBL's IL-15 ELISA measures both IL-15 alone and IL-15/IL15R α complexes. It can also quantitate IL-15 in healthy donor matrices. The standard in this assay is the IL-15/IL-15R heterodimer but because the antibodies used are specific for IL-15, the standard curve is calibrated to the IL-15 portion of the complex.

Note: Use shaker at 600 rpm speed for optimal assay results.

Visit the product page on PBL's website (<https://pblassaysci.com>) to view the technical supplement, including performance characterization and kit specifications.

CAUTION: Components should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

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PREPARATION OF REAGENTS

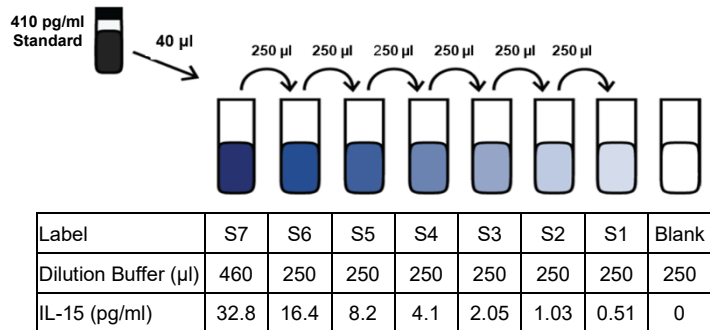
Wash Buffer: Wash Solution Concentrate may contain crystals; place bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution (e.g. Add 50 ml Wash Solution Concentrate to 450 ml distilled or deionized water). Mix thoroughly before use (**Note:** Prepare fresh Wash Buffer for each assay run.)

Human IL-15 Standard Curve Preparation:

Note: Use Dilution Buffer for dilution of serum/plasma samples.

- Pipette 40 μ l IL-15 standard into 460 μ l Dilution Buffer or Sample Matrix. Mix thoroughly by gently pipetting up and down 10 times.
- Label seven polypropylene tubes (S1 – S7).
- Add volume of Dilution Buffer or Sample Matrix to each tube as indicated in [Figure 1](#).
- Remove indicated amount from S7 and add to S6. Repeat to complete series to S1. *Change tips between each dilution.*

Figure 1: 7-Point Standard Curve Prepared in Dilution Buffer



Sample Preparation: Thaw frozen sample tubes to Room Temperature (RT) (22-25°C) in either tap water or between the fingertips. If samples require dilution, prepare using Dilution Buffer. Keep at RT (22-25°C) until step 1. Measurements in duplicate are recommended.

Antibody Concentrate: 15 minutes prior to use in step 3, dilute Antibody Concentrate in the volume of Concentrate Diluent as shown below. Keep at RT (22-25°C).

| Micro-plate Strips Used | 2 | 4 | 6 | 8 | 10 | 12 |
|---------------------------------|-----|-----|-----|-----|------|------|
| Antibody Concentrate (μ l) | 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 4, 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 (μ l) | 20 | 40 | 60 | 80 | 100 | 120 |
| 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 |
|---------------------------|----------------------|
| Plate/Plate Sealers | All other components |
| Wash Solution Concentrate | |
| Dilution Buffer | |
| Assay Buffer | |
| Concentrate Diluent | |
| HRP Diluent | |
| TMB Substrate Solution | |
| Stop Solution | |

- **Incubations:** Use plate sealers to cover the plate when directed. All incubations should be conducted in a closed chamber at 22-25°C (RT), keeping the plate away from drafts.
- **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.

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.

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

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

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

Cover with Plate Sealer and shake at 600 rpm at RT for 2 hours.

After 2 hours, empty plate contents and wash wells three times.

3. Add 100 µl of diluted **Antibody Solution** to each well. Cover with Plate Sealer and shake plate at 600 rpm at RT for 1 hour.

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

4. Add 100 µl of diluted **HRP Solution** to each well. Cover with Plate Sealer and shake plate at 600 rpm at RT for 30 minutes.

After 30 minutes, empty plate contents and wash wells four times.

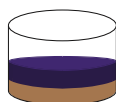
5. Add 100 µl of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 30 minutes. Do not use a Plate Sealer and **DO NOT SHAKE** during the incubation.

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

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

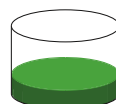
HUMAN IL-15 ELISA (41702) ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 4 hr



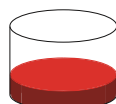
1. Add 50 µl Assay Buffer
2. Add 50 µl Standard, Sample or Blank
*Incubate 2 hr (shake at 600 rpm) at RT**

Aspirate and Wash 3x



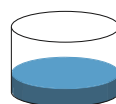
- Add 100 µl diluted Antibody Solution
*Incubate 1 hr (shake at 600 rpm) at RT**

Aspirate and Wash 3x

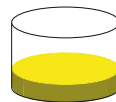


- Add 100 µl diluted HRP Solution
*Incubate 30 min (shake at 600 rpm) at RT**

Aspirate and Wash 4x



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



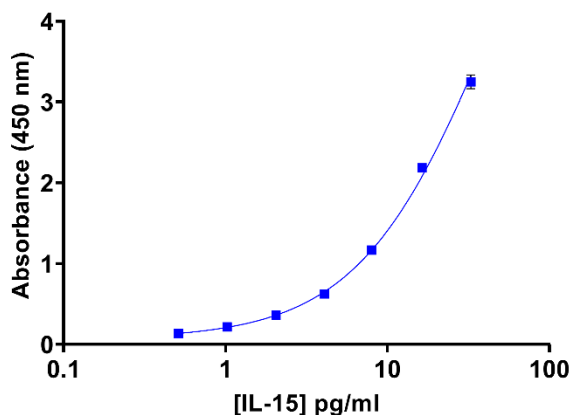
- Add 100 µl Stop Solution
Read plate within 2 min (450 nm)

***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. A 4-parameter logistic plot with $1/y^2$ weighted analysis is recommended for obtaining optimal fit of standard curve OD values. Blank ODs may be subtracted from the standards and sample ODs to eliminate background.

Figure 2: Typical Standard Curve in Dilution Buffer



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