



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: 7271

Expiration: July 31, 2021

Store all components at 2-8°C

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP350	K6202	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60	K6206	2 x 50 ml
Human IL-15 Standard, 410 pg/ml	SMP351-1	K6207	1 vial
Assay Buffer	SMP352-8	K6208	8 ml
Dilution Buffer	SMP021-60	K6209	55 ml
Antibody Concentrate	SMP354-1	K6211	1 vial
HRP Conjugate Concentrate	SMP056-240	K6212	1 vial
Concentrate Diluent	SMP024-15	K6210	15 ml
HRP Diluent	ASDHRP-15	625611	15 ml
TMB Substrate Solution	KET-15	200210D02	15 ml
Stop Solution	SCY-15	56334	15 ml

PREPARATION OF REAGENTS

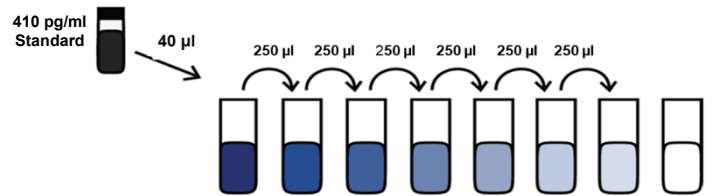
Wash Buffer: Wash Solution Concentrate may contain crystals; place bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution (e.g. Add 50 ml Wash Solution Concentrate to 450 ml distilled or deionized water). Mix thoroughly before use (**Note:** Prepare 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 µl IL-15 standard into 460 µl 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



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Dilution Buffer (µl)	460	250	250	250	250	250	250	250
IL-15 (pg/ml)	32.8	16.4	8.2	4.1	2.05	1.03	0.51	0

Authorization

Released by: _____

Date: October 15, 2020

INTRODUCTION

PBL's IL-15 ELISA measures both IL-15 alone and IL-15/IL15Rα 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.

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 (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 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 (µl)	20	40	60	80	100	120
HRP Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

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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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.

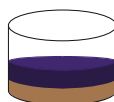
5. Add 100 µl of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 30 minutes. Do not use a Plate Sealer and **DO NOT SHAKE** during the incubation.

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.

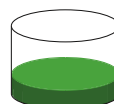
HUMAN IL-15 ELISA (41702) ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 4 hr



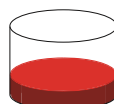
1. Add 50 µl Assay Buffer
2. Add 50 µl Standard, Sample or Blank
*Incubate 2 hr (shake at 600 rpm) at RT**

Aspirate and Wash 3x



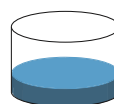
- Add 100 µl diluted Antibody Solution
*Incubate 1 hr (shake at 600 rpm) at RT**

Aspirate and Wash 3x

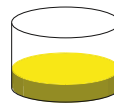


- Add 100 µl diluted HRP Solution
*Incubate 30 min (shake at 600 rpm) at RT**

Aspirate and Wash 4x



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



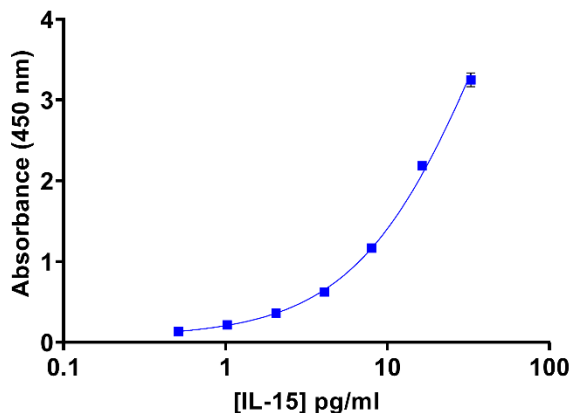
- Add 100 µl Stop Solution
Read plate within 2 min (450 nm)

***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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