VeriKine-HS™ Total 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:
Expiration:
Store all components at 2-8ºC

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

Authorization

Released by: _____

Date:

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.

CAUTION: Components 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 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.

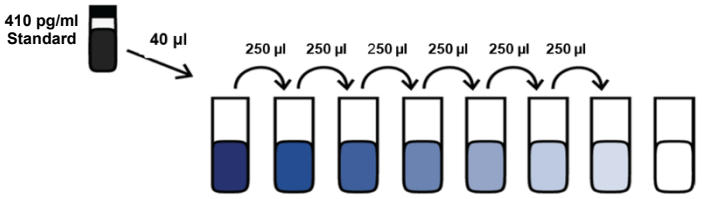
a. Pipette 40 µl IL-15 standard into 460 µl Dilution Buffer or Sample Matrix. Mix thoroughly by gently pipetting up and down 10 times.

b. Label seven polypropylene tubes (S1 – S7).

c. Add volume of Dilution Buffer or Sample Matrix to each tube as indicated in Figure 1.

d. 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

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 Dilution Buffer. Keep at RT (22-25ºC) 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 (22-25ºC).

Micro-plate Strips Used	**2**	**4**	**6**	**8**	**10**	**12**
Antibody Concentrate (µl)						
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 (22-25ºC).

Micro-plate Strips Used	**2**	**4**	**6**	**8**	**10**	**12**
HRP Conjugate Concentrate (µl)						
HRP 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/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 22-25ºC (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 2-8ºC. 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.

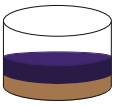
6. After 30 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

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

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

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

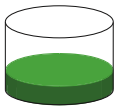
Total Time: 4 hr



1. Add **50 µl** Assay Buffer
2. Add **50 µl** Standard, Sample or Blank

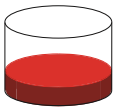
Incubate **2 hr** *(shake at 600 rpm) at RT**

Aspirate and Wash **3x**



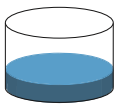
Add **100 µl** diluted Antibody Solution
Incubate **1 hr** *(shake at 600 rpm) at RT**

Aspirate and Wash **3x**

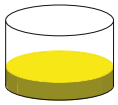


Add **100 µl** diluted HRP Solution
Incubate **30 min** *(shake at 600 rpm) at RT**

Aspirate and Wash **4x**



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



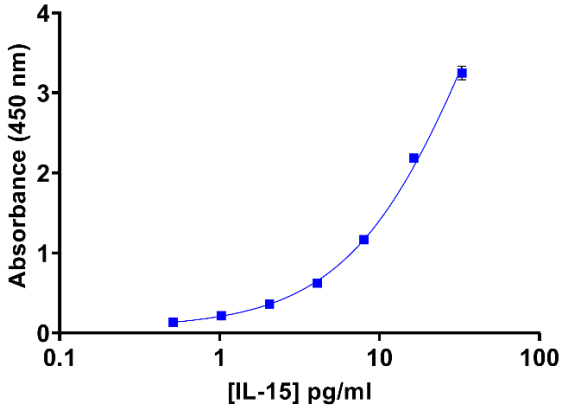
Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

***Note:** All incubations are at Room Temperature (RT) (22-25ºC)

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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