

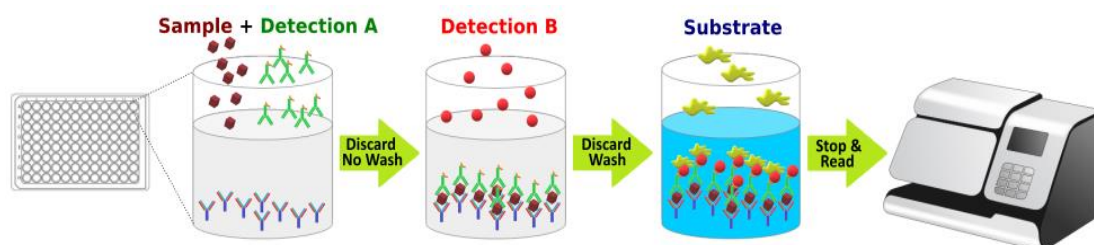
**INTRODUCTION**

Interleukin-3 (IL-3) is a pleiotropic broad-spectrum regulator of hematopoiesis that acts on numerous target cells, including primitive pluripotent hematopoietic stem cells, various lineage committed progenitors and mature hematopoietic cells. IL-3 enhances survival and stimulates the proliferation of hematopoietic stem cells and progenitor cells. IL-3 also regulates the functional activity of mature mast cells, megakaryocytes, basophils, eosinophils and macrophages.

The Fast Human IL-3 ELISA is a solid phase ELISA designed to measure human IL-3 levels in cell culture supernatants, serum, and plasma. The main feature is that **the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, not needing 4-5 hours (Fig. 1).** The detection range is from 15.6 to 1000 pg/mL. The levels of human IL-3 samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human IL-3 protein.

**PRINCIPLE OF THE ASSAY**

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific for human IL-3 is pre-coated onto a microplate. Standards or samples and a biotin conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well without washing and directly add Streptavidin-HRP into the complex. Following a wash, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is proportional to the amount of IL-3 bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

**Fig. 1: Assay Principle:**

**KIT CONTENT AND STORAGE CONDITIONS**

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human IL-3 Microplate	TBS3203A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human IL-3.	The unused wells can be stored in the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human IL-3 Standard	TBS3203B	30 ul of Recombinant human IL-3 protein (100ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3203C	2.1 ml of human IL-3 antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3203D	300 µl of Streptavidin-HRP	
Assay Diluent	TBS3203E	25 ml of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

The kit contains sufficient materials to run an ELISA on one 96 well plate.

**PRECAUTIONS**

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

**REAGENT PREPARATION**

**Bring all reagents to room temperature before use.**

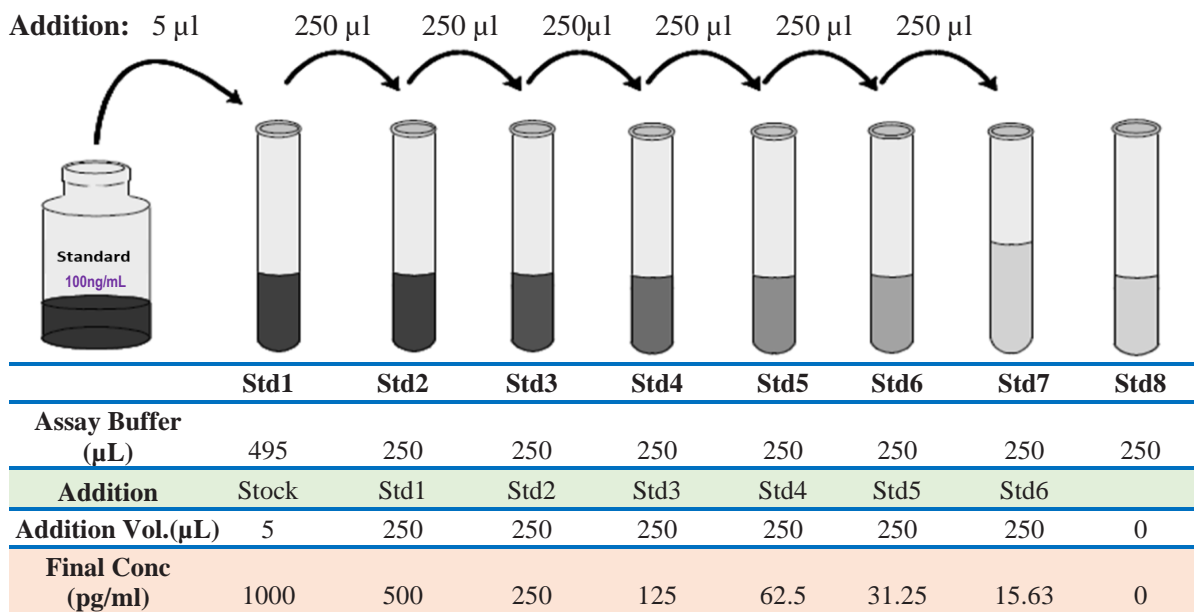
**Wash Buffer:** Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*)

**Detection B working solution preparation:** Add 240  $\mu$ L of **Detection B** streptavidin-HRP to 12 mL Assay Diluent to prepare Detection B working solution.

**Human IL-3 Standard Preparation:**

1. Label test tubes as #1 through #8. Pipet 495  $\mu$ L of 1x Assay Diluent into tube #1, and 250  $\mu$ L into tubes #2 to #8 as **diagram below (Fig. 2).**
2. Add 5  $\mu$ L of the Human IL-3 Standard stock solution (100ng/mL) by dilution of 100 times to tube #1 (1000pg/mL) and mix.
3. Make 2x serial dilutions of the standard using the 1000pg/mL standard solution in tube#1 from tube #2 through #7 with sequential transfer of 400  $\mu$ L to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 1000; 500; 250; 125; 62.5; 31.25 and 15.63 pg/mL. Tube# 8 is Standard 0.

**Fig. 2 Diagram for human IL-3 standard preparation**



**ASSAY PROCEDURE**

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Add 80  $\mu$ L of standard, sample, or control per well.
2. Add 20  $\mu$ L of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 1 hour.**
3. Aspirate each well (*no wash*). Invert the plate and blot it against clean paper towels.
4. Add 100  $\mu$ L of **Detection B** to each well. Incubate at **RT for 30min.**
5. Aspirate each well, and wash for 3 times by filling each well with 300  $\mu$ L Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100  $\mu$ L of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue. If the color is light, the incubation time can be longer.

7. Add 50µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

**CALCULATION OF RESULTS**

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

**TYPICAL DATA**

This standard curve ( $R^2=0.9925$ ) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

**SENSITIVITY**

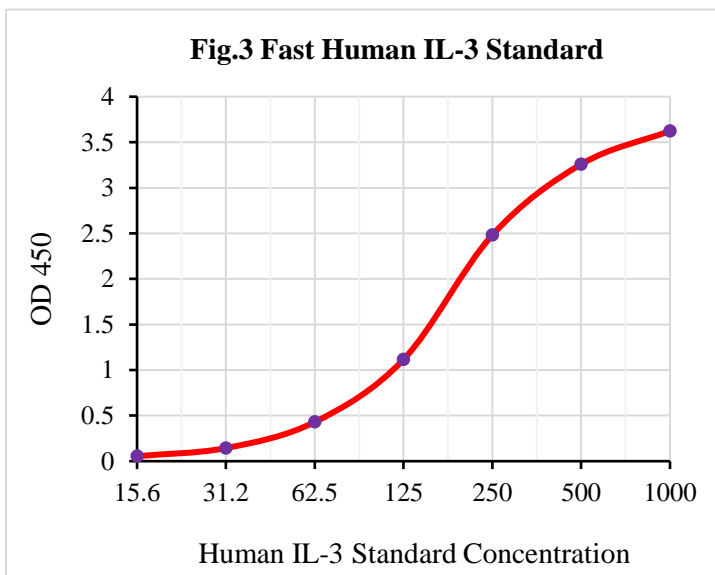
The minimum detectable dose (MOD) of human IL-3 is typically 3.0 pg/ml. The Intra-assay CV is 4.9% the Inter-assay CV is 8.1%.

**SPECIFICITY**

This assay recognizes natural and recombinant human IL-3. No cross-reactivity: human IL-1α; human IL-1β; Mouse IL-1β; Mouse IL-3; Mouse IL-4; Mouse IL-5.

**RELATIVE PRODUCTS**

- Human IL-1IL-1β ELISA (TBS3219)
- Human IL-2 ELISA (TBS3220)
- Human IL-4 ELISA (TBS3221)
- Human IL-6 ELISA (TBS3223)
- Human IL-7 ELISA (TBS3224)
- Human IL-8 ELISA (TBS3225)
- Human IL-10 ELISA (TBS3226)
- Human IL-13 ELISA (TBS3227)
- Human IL-17 ELISA (TBS3228)
- Human IL-22 ELISA (TBS3229)
- Human IFN-gamma ELISA (TBS3230)
- Human TGF- β1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1α ELISA (TBS3234)
- Human TNF-α ELISA (TBS3235)



**For research use only.**