INTENDED USE

The Interleukin-6 (IL-6) Test Kit is used for the determination of Interleukin-6 in human serum or plasma.

ANALYTICAL PRINCIPLE

Samples along with standards and controls are incubated in IL-6 antibody coated microwell. During incubation, IL-6 binds to antibody on the surface of the microwell. After incubation and washing, IL6-antibody complex remains on the surface of the microwell. IL6-antibody-HRP is allowed to bind to IL6-antibody complex. A wash step removes all unbound IL6-antibody-HRP. TMB is allowed to react with IL6-antibody-HRP on the microwell. The reaction is stopped and the microwells are read on a microplate reader. The level of IL-6 in the samples, standards, and controls are proportional to the intensity of color that is measured.

SPECIMEN REQUIREMENTS

Serum collected in red top tube, EDTA plasma, or SST tubes are acceptable. **Do not use excessively lipemic or hemolyzed samples.** Samples are stable for up to 2 days refrigerated (2-8°C) and up to 1.5 years frozen (-15 to -35°C).

REAGENTS

Precautions & Safety Notes

- WEAR LATEX GLOVES, FACE SHIELDS AND A LAB COAT WHEN HANDLING SPECIMENS AND OTHER HAZARDOUS REAGENTS
- FOR *IN VITRO* USE, POTENTIAL BIOHAZARDOUS MATERIAL. HANDLE ASSAY REAGENTS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.
- Sample Diluent, HRP Conjugate, TMB Solution, Calibrators, Controls, and 20X Wash Buffer are preserved with < 0.1% ProClin 300. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Name	Part Number	96 QTY	480 QTY
	IL-6 Test Kit	**IL6-3000**	**96 tests**	**480 tests**
A	Microwell Plate	**IL6-3001**	**96**	**5 x 96**
B	HRP Conjugate	**IL6-3002**	**10 mL**	**50 mL**
C1 – C6	Set of 6 Calibrators (0, 5.0, 20, 120, 240, 480 pg/mL) Lyophilized	**IL6-3003(A-F)**	**500 µl/vial**	**1.5 mL/vial**
D	Control Level 1 Lyophilized	**IL6-3004A**	**1 mL/vial**	**5 mL/vial**
E	Control Level 2 Lyophilized	**IL6-3004B**	**1 mL/vial**	**5 mL/vial**
TS	TMB Solution Keep away from light	**TMBS-1001**	**10 mL**	**50 mL**
SS	Stop Solution 1N Sulfuric Acid	**SSFEA-1001-5**	**5 mL**	**25 mL**
WB	20X Wash Buffer Concentrate	**WBFEA-1003**	**20 mL**	**100 mL**
SD	Sample Diluent	**SD-1004**	**10 mL**	**50 mL**
	Microwell film seal			

MATERIALS

Materials needed but not supplied:

- Variable Pipettors and Tips
- 12x75 mm disposable borosilicate glass culture tubes
- Refrigerator (for kit storage)
- Vortexer
- ELISA Plate Washer
- ELISA Plate Reader
- ELISA Plate Shaker

REAGENT PREPARATION

1X Wash Buffer Preparation

1. Add 1 unit of volume of 20X Wash Buffer Concentrate (**WB**) to 19 units of volume of DI water. For example, add **20 mL** 20X Wash Buffer Concentrate (**WB**) to **380 mL** of DI or purified water.
2. Mix the Wash Buffer Preparation well.

Calibrators Rehydration and Storage

1. Add **500 µL** of DI or purified water to each of the calibrators (IL6-3003A – IL6-3003F).
2. Cap each vial. Make sure each vial is capped well.
3. Incubate each vial at room temperature (15°C – 25°C) for 30 ± 2 minutes.
4. Gently invert each vial back and forth until the contents are completely dissolved.

- Before rehydration, store calibrators at 2°C - 8°C. Calibrators are stable until the expiration date on the label.
- After rehydration, calibrators are stable when stored at 2°C - 8°C for 6 weeks. Rehydrated calibrators are stable for up to 6 months when stored at -20°C ± 5 °C after rehydration.

Controls Rehydration and Storage

1. Add **1 mL** of DI or purified water to each of the controls (IL6-3004A, IL6-3004B).
2. Cap each vial. Make sure each vial is capped well.
3. Incubate each vial at room temperature (15°C – 25°C) for 30 ± 2 minutes.
4. Gently invert each vial back and forth until the contents are completely dissolved.

- Before rehydration, store controls at 2°C - 8°C. Controls are stable until the expiration date on the label.
- After rehydration, controls are stable when stored at 2°C - 8°C for 6 weeks. Rehydrated controls are stable for up to 6 months when stored at -20°C ± 5 °C after rehydration.

PROCEDURE PRECAUTIONS

- Bring reagents to room temperature before use.
- Use clean instruments & equipments.
- Microwells can be snapped to select exact number of wells.
- Unused microwells should be sealed in pouch.
- Handle microwells with care.
- Minimize air bubbles in microwells.
- Microwells should not contain liquid after the washing steps. To ensure that liquids are removed from microwells after washing steps, microwells can be tapped against clean paper towels.
- It is recommended to perform calibrator, controls, and unknowns in duplicates.

PROCEDURE

1. Arrange the required number of microwells on the microwell plate in accordance to the Microwell Assignment Table below.
2. Dispense **100 µL** of calibrators, controls (**C1 – C6, D, E**) and unknowns to microwells (**A**).
3. Cover microwells with microwell film seal.
4. Place on shaker at **480 RPM** for **1 hour and 30 ± 5 minutes**.
5. Wash each well **5 times** with **300 µL** of **1X Wash Buffer Preparation**. Tap the microwells against clean paper towels to ensure that no liquid remains
6. Dispense **100 µL** of HRP Conjugate (**B**) into each well.
7. Shake the wells for 30 seconds.
8. Incubate the wells for **1 hour ± 2 minutes** at **room temperature**.
9. Wash each well **5 times** with **300 µL** of **1X Wash Buffer Preparation**. Tap the microwells against clean paper towels to ensure that no liquid remains.
10. Dispense **100 µL** of TMB Solution (**TS**) to each well.
11. Incubate the wells for **30 minutes ± 1 minute** at **room temperature** and **away from light**.
12. Dispense **50 µL** of Stop Solution (**SS**) to each well.
13. Read the wells in a microwell reader at **450 nm**.

Microwell Assignment Table

Microwell	Name
A1 - A2	Calibrator 1
B1 - B2	Calibrator 2
C1 - C2	Calibrator 3
D1 - D2	Calibrator 4
E1 - E2	Calibrator 5
F1 - F2	Calibrator 6
G1 - G2	Control 1
H1 - H2	Control 2
A3 - end	unknowns

CALCULATIONS

A standard curve is used to calculate the concentration of unknowns.

1.Use the mean OD values of the calibrators to plot a standard curve on a linear graph. Plot absorbance on y axis and concentration on x axis.

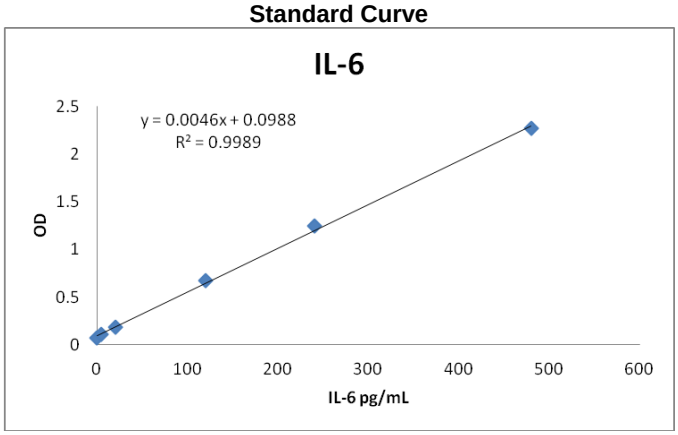
2. Plot the OD of the controls and unknowns using the standard curve to determine the concentration. Multiply the concentration found by the sample dilution factor, if a dilution was performed.

TYPICAL RESULTS

Standard Curve and Control Results			
pg/mL	**OD 1**	**OD 2**	**Mean OD**
0	0.076	0.07	0.073
5	0.114	0.111	0.1125
20	0.188	0.182	0.185
120	0.682	0.663	0.6725
240	1.26	1.226	1.243
480	2.324	2.216	2.27

Control	OD	Result
C1	0.132	10.50
C2	0.633	129.79

Standard Curve



POST PROCEDURE NOTES

- Abnormal and borderline results should be re-assayed for verification.
- Results that are higher than the highest standard can be diluted with Sample Diluent (**SD**) and re-assayed.

Manufacturer

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