Mouse IL-6 ELISA Kit

For the quantitative determination of mouse interleukin-6 (IL-6) concentrations in mouse serum, cell culture supernatant, and other biological fluids

Catalogue Number: MEC1008

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (03) mIL-6

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INTENDED USE

This Mouse IL-6 ELISA kit is to be used for the *in vitro* quantitative determination of mouse interleukin 6 (IL-6) concentrations in serum, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY.

INTRODUCTION

Interleukin 6 (IL-6) is a multifunctional cytokine secreted locally by a variety of cell types including T cells, B cells, monocytes/macrophages, endothelial cells, fibroblasts, hepatocytes, keratinocytes and astrocytes etc. IL-6 is up-regulated upon the binding of Toll-like receptors to antigen pattern ligands and by mitogenic stimulation during infection, acute phase reaction, trauma (especially burns) and malignancies. IL-6 secretion is significantly increased during physical excises. Also known as B-cell stimulatory factor-2 (BSF-2), IL-6 is an important cytokine for the differentiation of B-cells into immunoglobulin-secreting cells. In addition, IL-6 has been found to increase the number of platelets, influences cytotoxic T cell differentiation and activation, stimulate the differentiation and survival of neuronal cells, stimulate osteoclast formation, and induce terminal differentiation of M1 myeloid leukemic cells. IL-6 also stimulates the growth of hybridomas, plasmacytomas, myelomas, sarcomas, carcinomas, EBV-transformed B cells, keratinocytes, and mesangial cells. To melanoma cells and a few other cancer cells, IL-6 seemed to have an inhibitory effect.

In acute inflammatory phase, IL-6 causes increased body temperature by initiating synthesis of Prostaglandin E2 in hypothalamus and IL-6 stimulates the synthesis of acute phase proteins. IL-6 plays an anti-inflammatory role in acute reaction by decreasing the secretion of TNF-α and IL-1. The anti-inflammatory activity is regulated via the classic signal pathway by the direct binding of IL-6 to its membrane receptor. In chronic inflammation, IL-6 plays a pro-inflammatory role. The transition is mediated by soluble IL-6 receptor alpha (sIL-6Rα) via the Tran-signaling pathway.

Human IL-6 and murine IL-6 exhibit approximately 65% sequence homology at the nucleotide level, and 42% homology at the amino acid level.

PRINCIPLE OF THE ASSAY

This mouse IL-6 enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for mouse IL-6. Standards or samples are then added to the appropriate microtiter plate wells and incubated. Mouse IL-6, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound mouse IL-6 and other

components of the sample. In order to quantify the amount of mouse IL-6 present in the sample, a standardized preparation of biotin conjugated anti-mouse IL-6 detection antibody is added to each well. The detection antibody will bind to mouse IL-6 and be immobilized during the incubation. The plate is washed again and Avidin conjugated horseradish peroxidase (HRP) is added to each wells and incubated. Since Avidin has a very affinity to biotin, HRP will be linked indirectly to the detection antibody on the plate via the binding with of avidin to biotin. The microtiter plate is thoroughly washed to remove all unbound HRP and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain mouse IL-6 and will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of mouse IL-6 in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant/ urine testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus IL-6 concentration (pg/mL). The concentration of IL-6 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

This IL-6 ELISA is a 3 hour solid-phase immunoassay readily applicable to measure IL-6 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 12.5 to 800 pg/mL.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **IL-6 MICROTITER PLATE** (Part MEC08-1) ***96 wells***
   Pre-coated with anti-mouse IL-6 monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part MEC08-2) ***6 mL***
   Anti-mouse IL-6 antibody conjugated to Biotin.

3. **AVIDIN-HRP CONJUGATE** (Part MEC08-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase

4. **IL-6 STANDARD** (Part MEC08-4) ***2 vials***
   Recombinant mouse IL-6 (800pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part MEC08-5) ***25 mL***
   Animal serum with preservative. *For serum testing*.

6. **CALIBRATOR DILUENT II** (Part MEC08-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant/urine testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part MEC08-7) ***10 mL***
   Buffered solution with Urea-H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL required for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Mouse serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from mouse blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate mouse viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

- Avoid hemolytic, lipidic or turbid samples.
- Samples must be stored at -20°C (short term) or -70°C (long term) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- Serum and cell culture supernatant are to be thawed immediately before use.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

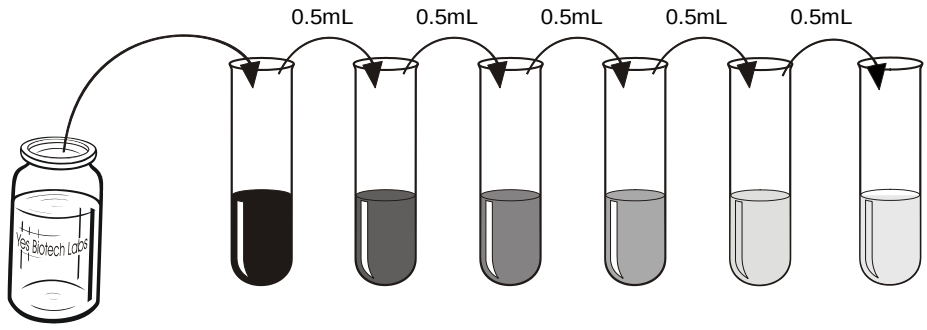
1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-6 Standard:**

a) Two vials of Standards are provided in this kit to allow both serum and cell culture supernatant testing. Reconstitute the mouse IL-6 Standard with either 1.0mL of Calibrator Diluent I (for serum testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 800pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (12.5pg/mL to 800pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-6 Standard will serve as the high standard (800pg/mL) and the Calibrator Diluent will serve as the zero-standard (0pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and mouse IL-6 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1- **0pg/mL** (S1)	**2A, 2B**	Standard 5- **100pg/mL** (S5)
1C, 1D	Standard 2- **12.5pg/mL** (S2)	**2C, 2D**	Standard 6- **200pg/mL** (S6)
1E, 1F	Standard 3- **25pg/mL** (S3)	**2E, 2F**	Standard 7- **400pg/mL** (S7)
1G, 1H	Standard 4- **50pg/mL** (S4)	**2G, 2H**	Standard 8- **800pg/mL** (S8)
3A, 12H	IL-6 samples		

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 25μL of Standard or sample to the appropriate well of the antibody pre-coated Microtiter Plate. Cover and incubate for 1 hour at room temperature.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Add 50μL Anti-IL-6 Biotin conjugate to each wells. Cover and incubate for 1 hour at room temperature.

5. Repeat wash procedure as described in Step 3

6. Dispense 100μL of Avidin-HRP conjugate to each well. Cover and incubate for 1 hour at room temperature. Prepare substrate solution refer to page 6 (Preparation of Reagent)

7. Repeat wash procedure as described in Step 3.

8. Add 100μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

9. Add 100μL Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION RESULT

The standard curve is used to determine the amount of mouse IL-6 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-6 concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IL-6 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-6 concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

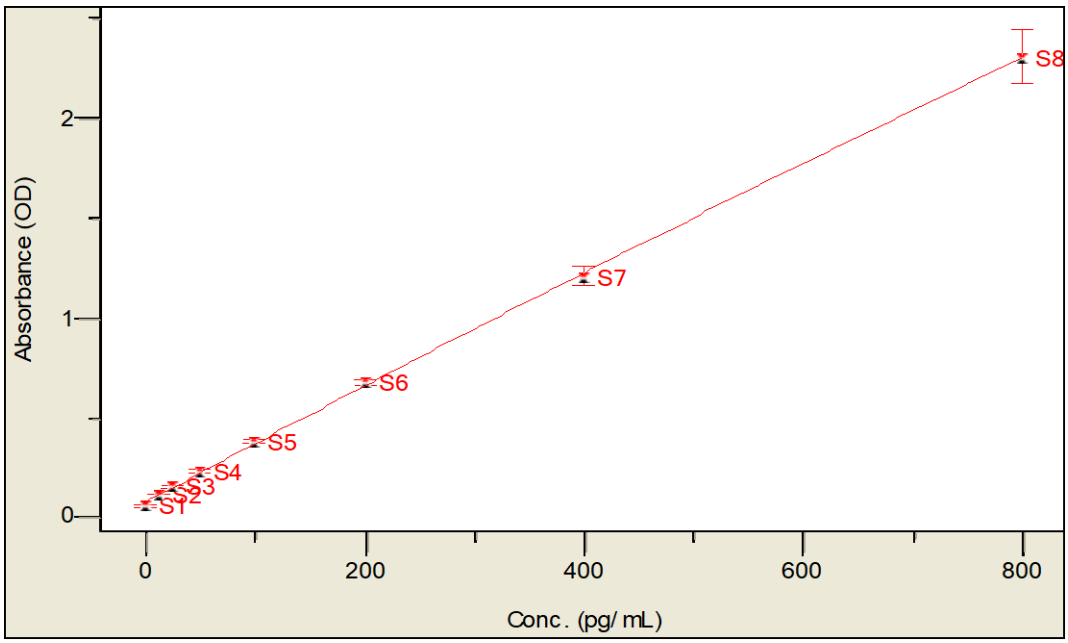
TYPICAL DATA

Results of a typical standard run of a mouse IL-6 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate user results.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

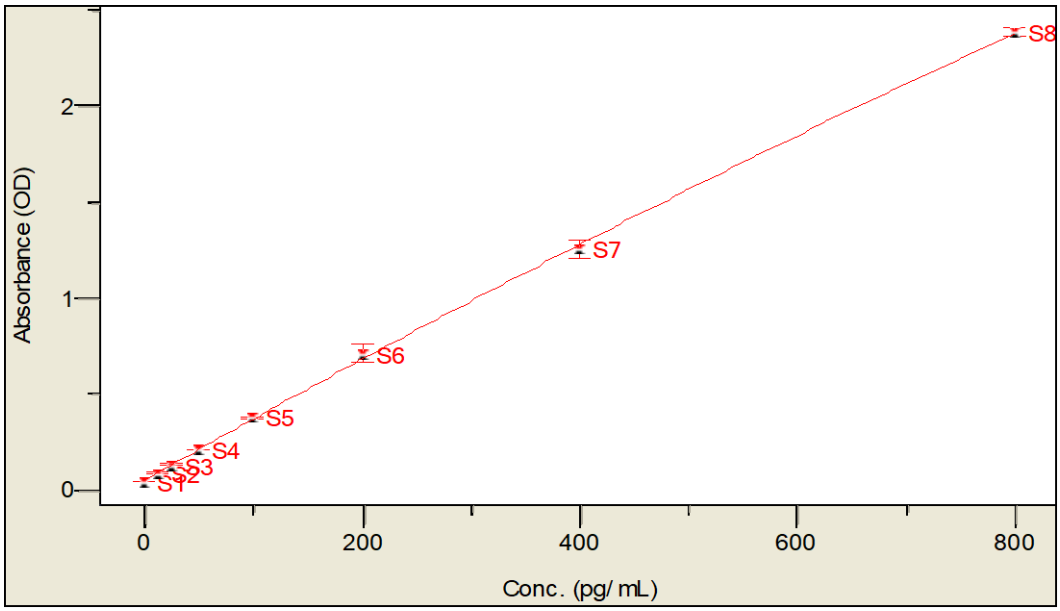
Standard (pg/mL)	**Mean O.D. (450 nm)**	**%CV**	**Zero Standard Subtracted** (Std.)-(S1)
0	0.063	6.73	0
12.5	0.121	3.51	0.058
25	0.159	2.67	0.096
50	0.237	4.77	0.174
100	0.388	1.82	0.325
200	0.6825	2.38	0.619
400	1.2135	3.90	1.147
800	2.3135	5.84	2.251



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	**Mean O.D. (450 nm)**	**%CV**	**Zero Standard Subtracted** (Std.)-(S1)
0	0.0515	1.37	0
12.5	0.0935	3.78	0.042
25	0.1385	2.55	0.087
50	0.2155	5.84	0.164
100	0.3845	1.66	0.333
200	0.7165	6.41	0.665
400	1.2595	3.87	1.208
800	2.3895	5.47	2.338



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 16 replicates in 1 assay.

Sample	**1**	**2**	**3**
N	16	16	16
Mean (pg/mL)	104.8	290.7	535.6
Coefficient of Variation (%)	2.98	4.89	2.79

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 8 different assays.

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
N	8	8	8
Mean (pg/mL)	103.2	291.2	543.1
Coefficient of Variation (%)	6.0	5.3	4.6

3. RECOVERY

The recovery of mouse IL-6 within cell culture medium and mouse serum was evaluated with mouse IL-6 spiked samples.

Sample Type	Average Recovery %	Range %
Cell culture media	93.5	91.7 –94.5
Mouse Serum	91.9	88-97.8

4. SENSITIVITY

The minimum detectable dose of mouse IL-6 was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of mouse IL-6 calculated from calibrate II diluted standard curve was <5.24pg/mL.

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CITATIONS

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