Life Technologies™
Your Molecular & Cell Technology Partner

Mouse IL6 ELISA Kit

to determine IL-6 concentration in mouse Serum, cell culture supernates and other biological cultures.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT36802EAYQ

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INTENDED USE

This kit is used to determine the IL-6 in the sample of mouse serum, cell culture supernates and related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.02

INTRODUCTION

Background

IL-6 is a multifunctional protein produced by lymphoid and non-lymphoid cells, and by normal and transformed cells, including T cells, monocyte/macrophages, fibroblasts, hepatocytes, vascular endothelial cells, cardiac myxomas, bladder cell carcinomas, myelomas, astrogliomas and glioblastomas. The production of IL-6 in these various cells is regulated, either positively or negatively, by a variety of signals including mitogens, antigenic stimulation, lipopolysaccharides, IL-1, TNF, PDGF and viruses. On the basis of its various activities, IL-6 has also been called interferon-β2 (IFN-β2), 26 kDa protein, B-cell stimulatory factor-2 (BSF-2), hybridoma/plasmacytoma growth factor, hepatocyte stimulating factor, cytotoxic T-cell differentiation factor, and macrophage-granulocyte inducing factor 2A (MGI-2A).

Assay principle

Mouse IL-6 ELISA Kit employs a two-site sandwich ELISA to quantitate IL-6 in samples. An antibody specific for IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-6 is added to the wells. After washing, proprietary EliKine™ Streptavidin-HRP conjugates is added to the wells. Following a wash to remove any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped and the intensity of the color is measured

Assay restrictions

• Do not mix or substitute reagents with those from other lots or sources.

• It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.

• If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.

• Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

• This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

PRODUCT INFORMATION

MATERIALS SUPPLIED

Mouse IL-6 microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with the antibody specific for Mouse IL-6.

Mouse IL-6 standard: Mouse IL-6 in a buffered protein base with preservatives; lyophilized.

Sample diluent: Diluent solution for reconstituted standard and samples. 5× liquid.

Mouse IL-6 detect antibody: Biotin-conjugated Mouse IL-6 detect antibody. 100× liquid.

Streptavidin-HRP: 100× liquid.

Assay buffer: Diluent solution for detection antibodies and Streptavidin-HRP. 5× liquid.

HRP substrate: TMB (Tetramethyl-benzidine) solution.

Stop solution: 2N sulfuric acid.

Wash buffer: PBS with 1% Tween-20; 20× liquid.

Plate covers.

OTHER SUPPLIES REQUIRED

• Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.

• Pipettes and pipette tips.

• Deionized or distilled water.

• Squirt bottle, manifold dispenser, or automated microplate washer.

• 500 mL graduated cylinder

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C for 1 year. Immediately after use remaining reagents should be returned to cold storage at 4˚C. Recommended storage instruction for opened/reconstituted kit components are listed below

Components	Storage conditions
Mouse IL-6 microplate	2-8 °C
Mouse IL-6 standard	2-8 °C
Mouse IL-6 detect Antibody	2-8 °C
Streptavidin-HRP	2-8 °C
Sample diluent	2-8 °C
Assay buffer	2-8 °C
Substrate	2-8 °C
Stop solution	2-8 °C
Wash buffer	2-8 °C

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator
2. Standard microplate reader capable of measuring absorbance at 450 nm.
3. Precision pipettes and disposable tips and Absorbent paper
4. Distilled or deionized water

PRECAUTIONS

Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.

Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

Technical hints

• When mixing or reconstituting protein solutions, always avoid foaming.

• To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

• Substrate solution should remain colorless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell culture supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20˚C. Avoid repeated freeze-thaw cycles. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 ˚C to avoid loss of bioactive Mouse CCL2(MCP-1). If samples are to be used within 24 hours, they may be stored at 2

to 8 ˚C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Pour entire contents (50 ml) of the Wash buffer (20×) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2 to 25 °C. Please note that Wash buffer (1×) is stable for 30 days.

Sample Diluent/ Assay buffer - Pour the entire contents (7 ml) of the Diluent (5×) into a clean 100 ml graduated cylinder. Bring to final volume of 35 ml with distilled water. Mix gently to avoid foaming. Store at 2 to 8 °C. Please note that the Diluent (1×) is stable for 30 days.

HRP substrate - The reagents should be ready within 15 minutes of use. Protect from light. 100 μL of the solution is required per well.

Standard - Reconstitute the Mouse IL-6 standard in 1mL of Sample Diluent for a concentration of 500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

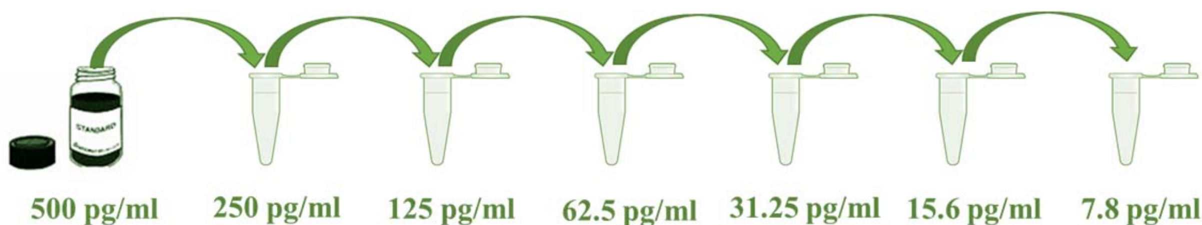
Mouse IL-6 detect antibody - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with Assay buffer in a clean plastic tube as needed according to the standards and samples. Detect antibody should be used within 30 minutes after dilution.

Streptavidin-HRP - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Streptavidin-HRP with Assay buffer in a clean plastic tube as needed according to the standards and samples. Streptavidin-HRP should be used within 30 minutes after dilution.

Sample dilution - If your samples need to be diluted, Sample diluent is used for dilution of serum/plasma samples, while cell culture medium is used for dilution of cell culture supernates.

ASSAY PROCEDURE

1. Dilution of standard solution: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:



250 pg/L	Standard No.6	500µl Original Standard + 500µl Standard diluents
125 pg/L	Standard No.5	500µl Standard No.6 + 500µl Standard diluentS
62.5 pg/L	Standard No.4	500µl Standard No.5 + 500µl Standard diluents
31.25pg/L	Standard No.3	500µl Standard No.4 + 500µl Standard diluent
15.6pg/L	Standard No.2	500µl Standard No.3 + 500µl Standard diluent
7.8pg/L	Standard No.1	500µl Standard No.2 + 500µl Standard diluent

2. Prepare all reagents and working standards as directed in the previous sections.

3. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

4. Add 100 µL of diluted standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.

5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or automatic washer. 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 µL of diluted Mouse IL-6 detect antibody to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in step 4.

8. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.

9. Repeat the aspiration/wash process for five times as in step 4.

10. Add 100 µL of TMB substrate solution to each well. Incubate for 15 minutes at room temperature. Protect from light.

11. Add 50 µL of Stop solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 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

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

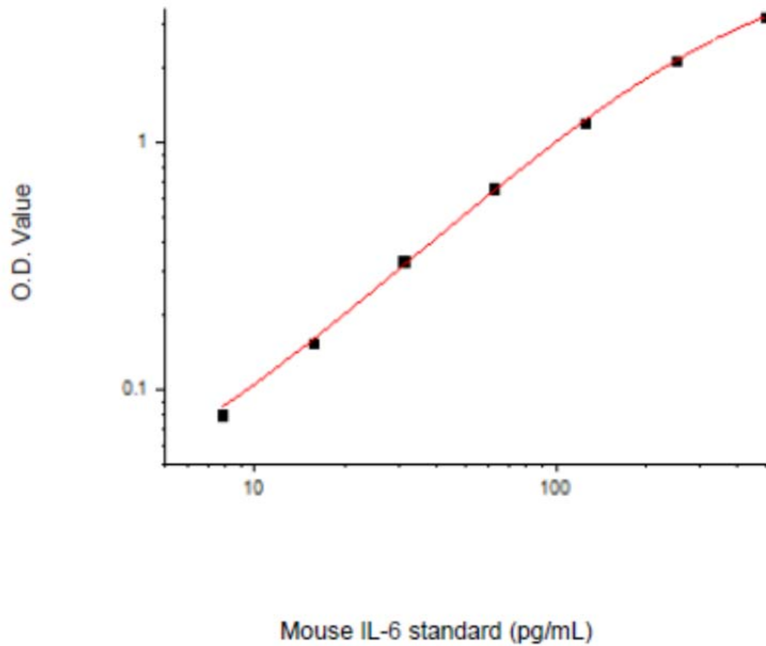
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Mouse IL-6 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.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical data

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Detection range: 7.8 pg/mL-500 pg/ml



Standard IL-6 (pg/mL)	Optical Density (450nm)	Average	Corrected
0	0.030	0.0285	——
	0.027		
7.8	0.112	0.108	0.0795
	0.104		
15.6	0.186	0.1835	0.155
	0.181		
31.25	0.369	0.3615	0.333
	0.354		
62.5	0.673	0.682	0.6535
	0.691		
125	1.263	1.2345	1.206
	1.206		
250	2.183	2.192	2.1635
	2.201		
500	3.316	3.2705	3.242
	3.225		

Precision

Intra-assay Precision (Precision within an assay)

Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)＜10%.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%)＜10%.

Recovery

The recovery of Mouse IL-6 spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.

The recovery ranged from 98% to 116% with an overall mean recovery of 106%.

Sensitivity

The minimum detectable dose (MDD) of Mouse IL-6 is typically less than 2 pg/mL. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Linearity

To assess linearity of the assay, samples containing and/or spiked with high concentrations of Mouse IL-6 were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Specificity

Mouse IL-6 ELISA Kit can be used to measure natural and recombinant Mouse IL-6. Mouse IL-6 ELISA Kit has high sensitivity and excellent specificity for detection of Mouse IL-6. No significant cross-reactivity or interference between Mouse IL-6 and analogues was observed.

VALIDITY & STORAGE: As per CoA.