EZElisa™ Mouse IL-2 ELISA Kit

Cat #: A-QEK00036

Size: 96wells

Storage: All reagents should be stored as indicated on the component label.

Product information

Introduction

IL-2 is a protein of 133 amino acids (15.4 kDa). It is produced mainly by T-cells expressing the surface antigen CD4 following cell activation by mitogens or allogens under physiological conditions. IL-2 displays significant anti-tumor activity for a variety of tumor cell types since it supports the proliferation and clonal expansion of T-cells that specifically attack certain tumor types. Mouse IL-2 is species-specific and is inactive on human cells.

Intended Use

EZElisa™ Mouse IL-2 ELISA Kit is specifically designed for accurate and quantitation of Mouse IL-2 from serum, plasma, cell culture supernatant or other bodily fluids. It is ready to use, accurate and sensitive.

Materials Provided

1. Microtiter Coated Plate (12 X 8 wells) – 1 plate
2. Recombinant Mouse IL-2 Standard lyophilized (0.5 ug/ml) – 1 vial
3. Mouse IL-2 Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. Mouse IL-2 Biotin Conjugated Detection Diluent – 12ml
6. (20X) Wash Buffer – 25ml
7. (5X) Assay Diluent – 10ml
8. TMB Substrate – 12ml
9. Stop Solution – 12ml
10. Instruction Manual

Materials to be provided by the End-User

1. Microplate Reader able to measure absorbance at 450 nm.

2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.

3. Deionized (DI) water.

4. Wash bottle or automated microplate washer.

5. Semi log graph paper or software for data analysis.

6. Tubes to prepare standard/sample dilutions.

7. Timer.

8. Absorbent paper

Handling/Storage

1. Store the kit components at 2-8°C.

2. Store lyophilized recombinant Standard at 2-8°C. Upon reconstitution, aliquot recombinant protein into polypropylene vials and store at -20°C as per assay requirements.

3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions..

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Procedure

Sample Preparation and Storage

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at

temperature < -20° C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at temperature < -20° C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at temperature < -20° C. Avoid repeated freeze/thaw cycles.

Reagent Preparation

Please refer to lot specific instructions for preparation of the reagents.

Assay Procedure

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicates. A standard curve is required for each assay.

2. Standard Preparation: Reconstitute the lyophilized vial with 10ul of Distilled water to generate a 0.5ug/ml main stock solution. Dilute 5 µl of reconstituted Standard (0.5 ug/ml) with 495 ul of Assay diluent (1X) to generate a 5 ng/ml middle stock solution. Prepare the Standard stock by diluting the middle stock as per the below table. Thus the Mouse IL-2 Standard Concentrations are 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.63 pg/ml. “Assay Diluent (1X) serves as the zero standard (0 pg/ml)”.

Standard Concentration	Standard No	Dilution Particulars
0.5 ug/ml	Standard,lyophilized	Original Standard provided in the Kit +10 ul Distilled water
5 ng/ml	Middle Stock	5 ul Original Standard+495 ul Assay diluent (1X)
500 pg/ml	Standard No.6	100 ul Middle Stock +900 ul Assay diluent (1X)
250 pg/ml	Standard No.5	500 ul Standard No.6+500 ul Assay diluent (1X)
125 pg/ml	Standard No.4	500 ul Standard No.5+500 ul Assay diluent (1X)
62.5 pg/ml	Standard No.3	500 ul Standard No.4+500 ul Assay diluent (1X)
31.25 pg/ml	Standard No.2	500 ul Standard No.3+500 ul Assay diluent (1X)
15.63 pg/ml	Standard No.1	500 Standard No.2+500 ul Assay diluent (1X)

3. Add 100µl/well of Standards and Samples to the plate, Seal plate and incubate for 2 hours at 37°C.

4. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

5. Add 100µl of diluted Detection Antibody solution to each well, seal plate and incubate for 1 hour at 37°C.

6. Wash plate 4 times with Wash Buffer (1X) as in step 4.

7. Add 100µl of diluted Streptavidin-HRP solution to each well, seal plate and incubate for 30 minutes at 37°C.

8. Wash plate 4 times with Wash Buffer (1X) as in step 4.

9. Add 100µl of TMB Substrate solution and incubate in the dark for 30 minutes at 37°C. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

10. Stop reaction by adding 100µl of Stop Solution to each well. Positive wells should turn from blue to yellow.

11. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Calculation of Results

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the xaxis and read the cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software may be preferred. Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

Performance Characteristics

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of „0" standard plus 2* SD. 10 replicates of „0" standards were evaluated and the LOD was found to 10 pg/ml.

Specificity:

The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Mouse IL-2.

Assay Range:

15.63 pg/ml to 500 pg/ml.

Precision:

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Linearity:

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Mouse IL-6 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
serum(n=5)	84-107%	87-108%	82-112%
EDTA plasma(n=5)	83-102%	83-115%	83-118%
heparin plasma(n=5)	83-99%	80-95%	82-93%

Safety Precautions

• This kit is for research use only. Follow the working instructions carefully.

• The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents

• Do not use or mix reagents from different lots.

• Do not use reagents from other manufacturers.

• Avoid time shift during pipetting of reagents.

• All reagents should be kept in the original shipping container.

• Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.

• Source materials maybe derived from Mouse body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

• Since the kit contains potentially hazardous materials, the following precautions should be observed.

- Do not smoke, eat or drink while handling kit material

- Always use protective gloves

- Never pipette material by mouth

- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

• In any case GLP should be applied with all general and individual regulations to the use of this kit.

SYMBOLS KEY

MTP	Coated Microtiter Plate (8x12 wells)
STD	Standard
BIOTIN AB	Biotinylated Antibody
HRP CONJ	Conjugate Horseradish Peroxidase
STD DIL	Standard Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.