EZElisa™ Mouse Interleukin 8 (IL-8/cxcl15) ELISA Kit

Cat #: A-QEK09061

Size: 96wells

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

Product information

Introduction

The EZElisa™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use

EZElisa™ Mouse Interleukin 8 (IL-8/cxcl15) ELISA Kit is used as an analytical tool for quantitative determination of Mouse Interleukin 8, IL-8 in serum, plasma and other biological samples.

Principle

The method employs sandwich ELISA technique. Mouse Interleukin 8 monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Mouse Interleukin 8, IL-8 present in the sample are bound by the antibodies. Biotin labeled IL-8 antibody is added and followed by Streptavidin HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Mouse Interleukin 8, IL-8 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided

1. Mouse IL-8 Antibody Coated Microtiter Plate (12 x 8 Wells)– 1 plate

2. Standard, Mouse IL-8 (concentrated, 240 ng/ml; lyophilized) – 2 vials

3. Mouse IL-8 Biotin Conjugated Detection Antibody (36 ug/ml; lyophilized)– 1 vial

4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial

5. (20X) Wash Buffer – 25 ml

6. (1X) Assay Diluent – 50 ml

7. TMB Substrate – 12 ml

8. Stop Solution – 12 ml

9. Instruction Manual

Materials to be provided by the End-User

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

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

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 main kit components at 2-8°C.

2. Store recombinant Standard and Detection Antibody at 2-8°C. Upon reconstitution, aliquot recombinant protein and detection antibody 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

Specimen Collection and Handling

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

1. 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.

2. 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.

3. 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. Standards Preparation: Reconstitute the lyophilized vial with 50 ul of Distilled Water to generate a 240 ng/ml. Dilute 33.33 ul of original Standard (240 ng/ml) with 966.67 ul of Assay diluent (1X) to generate a 8000 pg/ml top standard. Perform serial dilutions by using top 8000 pg/ml top standard as per the below table. Thus, the Mouse IL-8 standard concentrations are 8000 pg/ml, 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml and 250 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
240 ng/ml	Standard,lyophilized	Lyophilized Standard provided in the Kit + 75ul Distilled Water
8000 pg/ml	Standard No.6	33.33 ul Reconstituted Standard + 966.67 ul Assay Diluent (1X)
4000 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay Diluent (1X)
2000 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay Diluent (1X)
1000 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent (1X)
500 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent (1X)
250 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent (1X)

3. Add 100 ul of Standards and Samples to respective wells.

4. Seal plate and incubate at Room Temperature for 2 hours.

5. 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.

6. Add 100 ul of diluted Biotin Conjugated Detection Antibody to all wells.

7. Seal plate and incubate at Room Temperature for 2 hours.

8. Wash plate 4 times with Wash Buffer (1X) as in step 5.

9. Add 100 ul of diluted Streptavidin-HRP solution to each well, seal plate and incubate for 30 minutes at Room Temperature.

10. Wash plate 4 times with Wash Buffer (1X) as in step 5.

11. Add 100 ul of TMB Substrate solution and incubate in the dark for 30 minutes at Room Temperature. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

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

13. Read Absorbance at 450 nm within 30 minutes of stopping reaction.

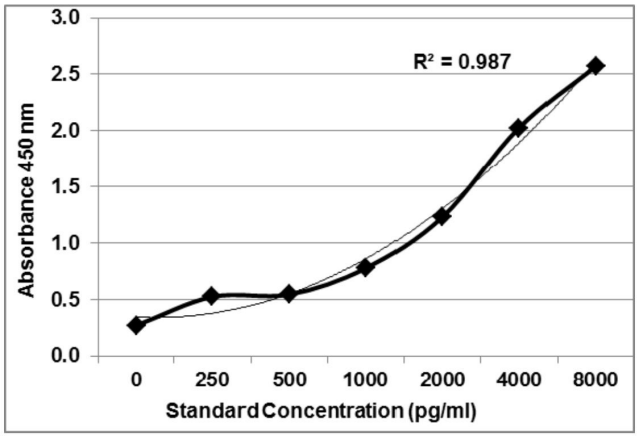
Calculation of Results

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 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 x-axis and read the cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data

Standard Concentration (pg/ml)	Means Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.273	--	--
250	0.526	299.2	119.7
500	0.546	404.1	80.8
1000	0.777	1054.7	105.5
2000	1.233	1971.0	98.6
4000	2.025	4027.3	100.7
8000	2.572	7964.3	99.6

Typical Graph



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 200 pg/ml.

Specificity:

The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Mouse IL-8.

Assay Range:

250 pg/ml to 8000 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-8 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 human 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	Mouse IL-8 Antibody Coated Microtiter Plate (12x8 wells)
STD	Mouse interleukin 8, IL-8 Standard, lyophilized
BIO CONJ	Biotin Conjugated Detection Antibody, lyophilized
STRP HRP	Streptavidin Horseradish Peroxidase
1X ASY DIL	(1X) Assay 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.