EZElisa™ Mouse Interleukin 17 alpha (IL-17A) ELISA Kit

Cat #: A-QEK08923

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 17 alpha (IL-17A) ELISA Kit is used as an analytical tool for quantitative determination of Mouse Interleukin 17A, IL-17A in serum, plasma and other biological samples.

Principle

The method employs sandwich ELISA technique. monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Mouse Interleukin 17A, IL-17A present in the sample are bound by the antibodies. Biotin labeled IL-17A 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 17A, IL-17A in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided

1. Mouse IL-17A Antibody Coated Microtiter Plate (8 x 12 wells) – 1 plate

2. Standard,Mouse IL-17A (concentrated, 800 pg/ml) – 0.5 ml

3. Biotinylated IL-17A Antibody – 1 ml

4. Streptavidin:HRP Conjugate – 6 ml

5. Standard Diluent – 3 ml

6. (20X) Wash Buffer – 25 ml

7. TMB Substrate – 12 ml

8. Stop Solution – 12 ml

9. Instruction Manual

Materials to be provided by the End-User

1. Microtiter Plate Reader able to measure absorbance at 450 nm.

2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul

3. Deionized (DI) water

4. Wash bottle or automated microplate washer

5. Graph paper or software for data analysis

6. Timer

7. Absorbent Paper

Handling/Storage

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

2. All the reagents and wash solutions should be used within 12 months from manufacturing date.

3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

2. For Research Use Only.

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.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.

2. Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

3. Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

4. Urine- Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

5. Cell Culture Supernatant- Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freezethaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.

6. Tissue Samples- Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

Reagent Preparation (all reagents should be diluted immediately prior to use)

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.

2. Bring all reagents to Room temperature before use.

3. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.

4. Standards Preparation: Dilute 120 ul of original Standard (800 pg/ml) with 120 ul of standard diluent to generate a 400 pg/ml Standard stock solution. Keep the standard for 15 mins with gentle agitation before making further dilutions.

Prepare the Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
800 pg/ml	Original Standard	Original Standard provided in the Kit
400 pg/ml	Standard No. 5	120 ul Standard Provided (800 pg/ml) + 120 ul Standard Diluent
200 pg/ml	Standard No. 4	120 ul Standard No.5 + 120 ul Standard Diluent
100 pg/ml	Standard No. 3	120 ul Standard No.4 + 120 ul Standard Diluent
50 pg/ml	Standard No. 2	120 ul Standard No.3 + 120 ul Standard Diluent
25 pg/ml	Standard No. 1	120 ul Standard No.2 + 120 ul Standard Diluent

Procedural Notes

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.

2. High Dose Hook Effect may be observed in samples with very high concentrations of Mouse Interleukin 17A, IL-17A present in the sample.

3. Mouse Interleukin 17A, IL-17A concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.

4. Avoid assay of Samples containing sodium azide (NaN3), as it could destroy the HRP activity resulting in under-estimation of the amount of Mouse Interleukin 17A, IL-17A.

5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.

6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.

7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.

8. The plates should be read within 30 minutes after adding the Stop Solution.

9. Make a work list in order to identify the location of Standards and Samples

Assay Procedure

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add 50 ul prepared Standards to respective standard wells.

3. Add 40 ul Samples to respective sample wells.

4. Pipette 10 ul Biotinylated IL-17A Antibody to respective sample wells.

Note: Do not add Biotinylated IL-17A Antibody to standard wells. The standards provided in the kit are preoffered as a complex of the standard and the biotin antibody for ease-of-use.

5. Pipette 50 ul Streptavidin:HRP Conjugate to all wells. Mix well.

6. Cover the plate with a sealer and incubate for 60 minutes at 37°C.

7. Aspirate and wash plate 4 times with diluted 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.

8. Pipette 100 ul TMB Substrate to all wells.

9. Incubate the plate at 37°C for 10 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette 100 ul of Stop Solution to all wells. The wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Mouse Interleukin 17A, IL-17A concentrations, find the unknown's 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 Mouse Interleukin 17A, IL-17A Concentration.If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit

This kit has been validated. Please view the details herein below.

Standard Calibration Range:

25 pg/ml – 400 pg/ml

Sensitivity and Limit Of Quantification:

It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 2.365 pg/ml.

Specificity:

The antibodies used in this kit are monoclonal antibodies specific for Mouse Interleukin 17A, IL-17A.

Precision:

Intra-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Mouse Interleukin 17A, IL-17A were

tested in triplicate respectively. The Intra-Assay was found to be <15% Inter-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Mouse Interleukin 17A, IL-17A were tested in triplicate on two plates respectively on two consecutive days. The Inter-Assay was found to be <18%.

The Cumulative Variance % was calculated as CV (%) = SD/mean x 100 [SD=standard deviation]

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.