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User's Manual

Anti-Tocilizumab ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Anti-Tocilizumab ELISA is used as an analytical tool for quantitative determination of AntiTocilizumab in serum, plasma and cell culture supernatant.

General Description

Tocilizumab, also known as atlizumab, is an immunosuppressive drug, mainly for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis, a severe form of arthritis in children. It is a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R). Interleukin 6 (IL-6) is a cytokine that plays an important role in immune response and is implicated in the pathogenesis of many diseases, such as autoimmune diseases, multiple myeloma and prostate cancer. It was developed by Hoffmann–La Roche and Chugai. Anti-Drug Antibodies (ADA) may induce unwanted side effects in biopharmaceuticals. Hence, ADA has been subjected to increase in scrutiny by the regulatory authorities using immunogenicity safety studies. ADA has been observed in pre-clinical and clinical studies, resulting in significant changes in toxicology, pharmacokinetics and efficacy. These effects result from the generation of drug-induced (neutralizing) autoantibodies against Tocilizumab and can be responsible for allergic reaction, or even anaphylactic shock. This ELISA kit detects antibodies for Anti-Tocilizumab and may be used for monitoring immunogenicity.

Principles of Testing

The method employs the quantitative sandwich enzyme immunoassay technique. Tocilizumab is pre-coated onto microwells. Samples and standards are pipetted into microwells and antibodies to Tocilizumab present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated Tocilizumab is pipetted and incubated. After washing microwells in order to remove any nonspecific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Anti-Tocilizumab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Reagents And Materials Provided

1. Tocilizumab Coated Microtiter Plate (12x8 wells) – 1 plate
2. Anti-Tocilizumab Standard, (0.5 ml/vial) – 0, 10, 20, 40, 80, 160, 320 and 640 ng/ml
3. Tocilizumab:HRP Conjugate – 12 ml
4. Assay Diluent – 6 ml
5. Sample Diluent – 50 ml
6. Wash Buffer (20X) – 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25µl to 1000µl
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Semi-Log graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Storage

1. All reagents should be stored at 2°C to 8°C for stability.
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.

Specimen Collection And Preparation

1. Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.
2. For Cell Culture Supernatant – If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80^{0}C. Avoid repeated freeze-thaw cycles.
3. Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.
4. Test Sample preparation - Samples have to be diluted 1:10 to 1:100 (v/v), e.g. for 1:100 (5 µl sample + 495 µl sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Reagent Preparation

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 50 ml of 20X Wash Buffer in 950 ml of DI water.

Assay Procedure

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standardn curve is required for each assay. All steps must be performed at 37°C.

2. Pipette out 50 µl of Assay Diluent in each well.
3. Add 100 µl of Standards or Samples into the respective wells.
4. Cover the plate and incubate for 60 minutes at 37°C.
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.
6. Pipette without delay in the same order 100 µl of Tocilizumab: HRP Conjugate into each well.
7. Cover the plate and incubate for 60 minutes at 37°C.
8. 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.
9. Add 100 µl of TMB Substrate in each well.
10. Incubate the plate at 37°C for 15 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
11. Pipette out 100 µl of Stop Solution. Wells should turn from blue to yellow in color.
12. Read the absorbance at 450 nm with a microplate reader.

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.

Calculation

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log 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 Anti-Tocilizumab 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 Anti-Tocilizumab 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.

- If the absorbance value is equivalent or higher than the 640 ng/ml standard.

Precision

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (10ng/ml), medium (80ng/ml) and high (640ng/ml)

concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

Detection Limit

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 be less than 10 ng/ml.

Precautions

1. This kit is for in vitro use only. Follow the working instructions carefully.
2. The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
3. Do not use or mix reagents from different lots.
4. Do not use reagents from other manufacturers.
5. Avoid time shift during pipetting of reagents.
6. All reagents should be kept in the original shipping container.
7. 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.
8. 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.
9. Since the kit contains potentially hazardous materials, the following precautions should be observed.
10. Do not smoke, eat or drink while handling kit material.
11. Always use protective gloves.
12. Never pipette material by mouth.
13. Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
14. In any case GLP should be applied with all general and individual regulations to the use of this kit.