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Datasheet for ABIN4886398
Tocilizumab ELISA Kit

Overview

Quantity:	96 tests
Target:	Tocilizumab
Reactivity:	Human, Mouse, Rat
Method Type:	Sandwich ELISA
Detection Range:	1.56-50 ng/mL
Minimum Detection Limit:	1.56 ng/mL
Application:	ELISA

Product Details

Purpose:	Quantification of Tocilizumab in biological matrices
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Tocilizumab (Actemra)
Cross-Reactivity (Details):	hIgG1, Rituximab, and Infliximab prepared at 250 ng/mL were assayed and exhibited no cross-reactivity or interference.
Sensitivity:	1.5 ng/mL
Components:	Coated microtiter plate, 96 wells Calibrator diluent. - 1.8ml Calibrator 12ul

Product Details

10X wash buffer - 25ml
Assay buffer - 50ml
1000X detection reagent - 17ul
TMB - 12ml
TMB stop solution - 12ml
Plate sealers - 3

Material not included: Precision pipettes calibrated to deliver 5-1000µL
Multi-channel pipette calibrated to deliver 50-200µL
Plate shaker
Disposable tips
Vortex-Mixer
Distilled or de-ionized water
Microplate reader capable of reading 450nm with background subtrac

Target Details

Target: Tocilizumab

Background: Tocilizumab (Actemra®) is an immunosuppressive humanized monoclonal antibody drug, mainly for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis, a severe form of arthritis in children. Tocilizumab binds the interleukin-6 receptor (IL-6R) thus blocking the signaling caused by interleukin 6 (IL-6). IL-6 is a cytokine that plays an important role in immune response and is implicated in the pathogenesis of many diseases.

Gene ID: 3570

Application Details

Application Notes: Optimal working dilution should be determined by the investigator.

Sample Volume: 15 µL

Assay Time: 2.5 h

Plate: Pre-coated

Protocol: The Tocilizumab ELISA kit is designed to measure free Tocilizumab with high specificity and sensitivity . This assay employs the sandwich enzyme immunoassay technique. A precoated anti-Tocilizumab 96 well plate is provided. Calibrator, quality control samples and test samples are pipetted into the appropriate wells. Tocilizumab present in biological matrices is bound by

Application Details

the immobilized capture antibody. After washing away any unbound substances, enzyme linked detection antibody is added to the wells. The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of Trastuzumab present in test samples and the concentration is calculated from the standard series.

Reagent Preparation: Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label. 1. Wash Buffer (1X) Preparation: Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 20 mL concentrate to 180 mL ultra-pure water). Mix well. 2. Detection Reagent (1X) Preparation: Dilute detection reagent with assay buffer 1/1000 before use (for example add 11 μ L concentrate to 11 mL of assay buffer). Mix well. 3. Preparation of Calibrators: Prepare calibrators with concentrations ranging from 2,500 ng/mL to 78 ng/mL. The following is an example calibrator curve.

Sample Collection: This kit is compatible with EDTA-plasma, heparinplasma and serum samples. Samples can be stored at or below -20 °C for up to 1 year.

Sample Preparation: Dilute calibrators and test samples 1/50 with assay buffer (for example add 5 μ L of prepared calibrator or sample to 245 μ L of assay buffer). Mix well. Do not store diluted samples.

Assay Procedure: This assay employs the sandwich enzyme immunoassay technique. Anti- Tocilizumab is coated onto a 96 well microplate. Calibrator and test samples are pipetted into the appropriate wells. Tocilizumab present in biological matrices is bound by the immobilized anti- Tocilizumab antibody. After washing away any unbound substances, enzyme linked antiTocilizumab antibody is added to the wells. This antibody is developed and purified specifically against Actemra® (domain residing in Fc portion of the Actemra® molecule). The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of Tocilizumab present in test samples. The color development is stopped and the intensity of the color is measured

Calculation of Results: 1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used. 2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample. 3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately with calibrator diluent and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

Assay Precision: Precision: Precision was determined by analyzing 6 replicates of serum spiked with 500ng/mL

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Tocilizumab on 6 different occasions. Intra-assay coefficient of variation (CV) < 10%. Inter-assay CV < 10%.

Recovery: 250 ng/mL of Tocilizumab was spiked in 10 lots of human serum. Recovery ranges are from 88-112% with an average recovery of 104%.

Restrictions: For Research Use only

Handling

Preservative: Without preservative

Precaution of Use: Read manual completely before beginning

Storage: -20 °C

Storage Comment: Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Expiry Date: 12 months