Anti-Tocilizumab (Human) ELISA Kit

Catalog Number KA6252

96 Assays

Version: 01

Intended for research use only

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Introduction

Product Description

This ELISA kit is used for in vitro qualitative determination of antibody against Tocilizumab in serum and plasma.

Background

Tocilizumab is a recombinant, humanized, anti-human interleukin 6 (IL-6) receptor monoclonal antibody. Tocilizumab is mainly used for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis, a severe form of arthritis in children.

Principle of the Assay

Anti-Tocilizumab (Human) ELISA Kit is designed to detect antibody against Tocilizumab with high specificity and sensitivity in serum and plasma samples. anti-Tocilizumab ELISA is based on the sandwich ELISA principle. Controls and samples are incubated in the microtiter plate coated with the drug Tocilizumab. After incubation, the wells are washed. Then, HRP conjugated probe is added and binds to Tocilizumab antibodies captured by the drug Tocilizumab on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of Tocilizumab antibodies in the sample or controls. The results can be evaluated with using cut-off value.

General Information

Materials Supplied

List of component

Component	Amount
Micro ELISA Plate	1 plate
Positive Control	0.3 mL
Negative Control	1 mL
Assay Buffer	12 mL
Peroxidase Conjugate	12 mL
TMB substrate (Avoid light)	12 mL
Stop Solution	12 mL
Wash buffer (20X)	50 mL
Plate sealers	2 slices

Storage Instruction

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm
- ✓ Precision pipettes with disposable tips
- ✓ Clean eppendorf tubes for preparing standards or sample dilutions
- ✓ Absorbent paper

Precautions for Use

For research use only. Not to be used on humans.

Assay Protocol

Reagent Preparation

Note: Before using the kit, spin tubes and bring down all components to the bottom of tubes

- ✓ Wash Buffer:
  Dilute the 20X Wash Buffer to 1X solution in ddH_2O (10 mL of Wash Buffer stock to 190 mL of ddH_2O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

Sample Preparation

- ✓ Sample type: Human serum and plasma.
- ✓ The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

Assay Procedure

Note: Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.

1. Prepare all reagents, samples and standards as instructed in section of Reagent Preparation and Sample Preparation.
2. Pipette 100 µL of Assay Buffer into each of the wells to be used.
3. Add 10 µL of negative control (2 wells), positive control (1 well), and samples into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
4. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
5. Add 100 µL of Peroxidase Conjugate into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
6. Discard the solution and wash the wells as step 3.
7. Add 100 µL of TMB substrate solution and incubate the plate in dark at RT for 20 minutes
8. Add 100 µL of Stop solution to stop the reaction
9. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

Data Analysis

Calculation of Results

- ✓ For the run to be valid, the $OD_{450/650}$ nm of Positive Control (Standard A) should be ≥ 1.500 and the $OD_{450/650}$ nm of each Negative Control should be <0.150, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- ✓ The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the negative controls by 3
  - If "Sample $OD_{450/650}$ / the mean Negative Control $OD_{450/650}$" is < 3, the sample is NEGATIVE for Antibody to Tocilizumab.
  - If "Sample $OD_{450/650}$ / the mean Negative Control $OD_{450/650}$" is ≥ 3, the sample is POSITIVE for Antibody to Tocilizumab.

  Note: The cut-off information provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users.

Performance Characteristics

- ✓ Cross Reactivity: Tocilizumab infusion camouflages/masks the presence of antibody to Tocilizumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of anti-drug-antibodies. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Tocilizumab.

Resources

Plate Layout

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7								
6								
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3								
2								
1								
	A	B	C	D	E	F	G	H