Technical Manual

Ocrelizumab ELISA Kit

- **Catalogue Code: SBRS2022**
- **Sandwich Principle**
- **Research Use Only**

Contents

Key features and Sample Types

Aliases:

Ocrelizumab

Detection method:

Sandwich-based (Colorimetric)

Sample Types:

Cell Culture Supernatants, Plasma, Serum

Reactivity:

Human

Storage & Expiry

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

Introduction

How do our ELISA kits work?

The Ocrelizumab ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of drug antibody (DA) in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for the DA coated on a 96-well plate. Standards and samples are pipetted into the wells and DA present in the sample is bound to the wells by the immobilized antibody. The wells are washed and HRP-conjugated detection antibody is added. The wells are again washed, and a TMB substrate solution is added to the wells and color develops in proportion to the amount of DA bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-Well Kit	Storage
1	Anti-DA Microplate	8 x 12	1 month at 4°C*
2	Wash Buffer Concentrated (20X)	25ml	1 month at 4°C
3	DA Standard	2 vials	1 month at 4°C
4	Assay DiluentB (5X)	15 ml	1 month at 4°C
5	HRP-conjugated Anti-DA Detection Antibody	2 vials	5 days at 4°C.
6	TMB One-Step Substrate Reagent	12ml	N/A
7	Stop Solution	8ml	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 litre graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare the positive control or sample dilutions.

Reagent Preparation

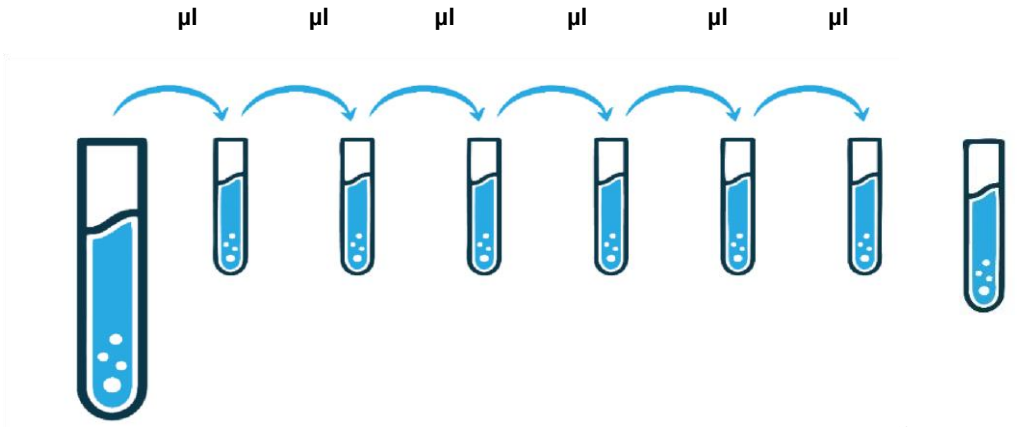
1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. Sample dilution: 1X Assay Diluent B (Item E) should be used for dilution of serum, plasma, and cell culture supernatant samples.

Note: Levels of Antibody Drug may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

3. Preparation of standard: Appropriate standard preparation will be determined during the development process.

DILUTION SERIES



Diluent Volume	Standard DA µl +						
		µl	µl	µl	µl	µl	µl	µl
Conc.								0

4. If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
5. Appropriate Detection Antibody preparation will be determined during the development process.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500 rpm.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

Assay Procedure Summary

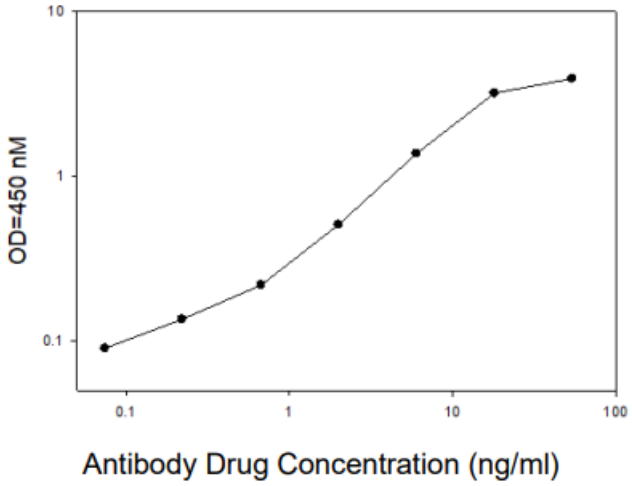
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2 hours at room temperature.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
5. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. Sensitivity

The minimum detectable dose of Antibody Drug will be determined during the development process.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer)

C. Spiking and Recovery

Recovery was determined by spiking various levels of Human TIGIT into the sample types listed below. Mean recoveries are as follows:

D. Linearity

Linearity will be determined in serum, plasma and cell culture media during the development process.

E. Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Specificity

This ELISA antibody pair detects Antibody Drug. Other targets not determined.

Troubleshooting

Problem	Causes	Solutions
Low signal in samples	• Sample concentration is too low • Improper preparation of detection antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background) • Check pipettes and ensure correct preparation
Poor standard curve	• Inaccurate pipetting Improper standard dilution	• Check pipettes • Briefly centrifuge Standard and dissolve the powder thoroughly by gently mixing
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store your standard at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light. • Add stop solution to each well before reading plate

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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