Omalizumab ELISA Kit

Enzyme Immunoassay kit for the quantification of Omalizumab in serum and plasma.

Catalog number: ARG82285

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Omalizumab, sold under the trade name Xolair, is a medication originally designed to reduce sensitivity to allergens. It has been used to try to control severe allergic asthma, which does not respond to high doses of corticosteroids and less widely for chronic spontaneous urticaria.

Omalizumab is a recombinant DNA-derived humanized IgG1k monoclonal antibody produced by cells of an adapted Chinese hamster ovary (CHO) cell line. Omalizumab specifically binds to free human immunoglobulin E (IgE) in the blood and interstitial fluid and to membrane-bound form of IgE (mIgE) on the surface of mIgE-expressing B lymphocytes. Unlike an ordinary anti-IgE antibody, it does not bind to IgE that is already bound by the high affinity IgE receptor (FcεRI) on the surface of mast cells, basophils, and antigen-presenting dendritic cells. [Wikipedia: Omalizumab]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. The reactant specific for omalizumab has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any omalizumab present is bound on the plate. After washing away any unbound substances a HRP-labeled reagent is added to each well and incubate to bind to omalizumab. After washing away any unbound reagents, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of omalizumab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm. The concentration of omalizumab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Omalizumab reactant -coated microplate	8 X 12 strips	4°C
Standard 0-4 (0, 30, 100, 300, 1000 ng/ml)	5 X 0.3 ml (Ready to use)	4°C
Control High	0.3 ml (Ready to use)	4°C
Control Low	0.3 ml (Ready to use)	4°C
HRP-conjugated	12 ml(Ready to use)	4°C
Assay Buffer	50 ml (Ready to use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml (Ready to use)	4°C (Protect from light)
STOP solution	12 ml (Ready to use)	4°C
Plate sealer	2 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm (optional: read at 650 nm as reference wave length)
- Pipettes and pipette tips
- Deionized or bidistilled water
- Graduated cylinder and beaker
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- For professional use only.
- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Once the foil bag has been opened, care should be taken to close it tightly.
- Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.
- Briefly spin down the HRP-conjugate reagent before use.
- The TMB substrate should be stored in the dark due to its sensitivity against light. The TMB substrate should be avoided contact with metals.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Use the Wash Buffer contained in this kit only. Insufficient washing may lead to the failure in measurement.
- Remove the Wash Buffer completely by tapping the microtiter plate on paper towel. Do not wipe wells with paper towel.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time.

- Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
- Change pipette tips between the addition of different reagent or samples.
- Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel micropipette for pipetting of solutions in all wells.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately. Sample can be aliquoted & stored samples at 4°C for up to 2 days or -20°C up to 6 month. Keep away from heat or direct sun light and avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately. Sample can be aliquoted & stored samples at 4°C for up to 2 days or -20°C up to 6 month Keep away from heat or direct sun light and avoid repeated freeze-thaw cycles.

Note:

a) Omalizumab (Xolair®) infusion camouflages/masks the presence of antibody to Omalizumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of Omalizumab. Matriks Biotek

Laboratories propose to obtain blood sample just before the infusion of Omalizumab (Xolair®) or at least 2 weeks after the infusion of Omalizumab (Xolair®)

b) Do not use grossly hemolytic, icteric or grossly lipemic specimens.

c) Samples containing sodium azide should not be used in the assay.

d) Samples appearing turbid should be centrifuged before testing to remove any particulate material.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into bidistilled water to yield 1X Wash buffer (E.g.: 50 ml 20X Wash buffer + 950 ml bidistilled water). The prepared wash buffer should be stored in 2-8°C and used within 2 weeks after dilution. Prepare the 1 X Wash Buffer before starting assay procedure.
- Samples: For serum/plasma samples we might suggest to be diluted at 1:200 dilution with Assay Buffer before assay. If the initial assay found samples contain omalizumab higher than the highest standard, the samples can be diluted with Assay Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, (18-25°C) and mixed it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **Assay Buffer** into each of the wells to be used.
3. Add **10 µl** of **standards, controls and diluted samples** into wells. Cover the plate with adhesive foil and incubate for **30 min at room temperature (18-25°C).**
4. Remove adhesive foil and aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **HRP-conjugate reagent** into each well. Cover wells with adhesive foil and incubate for **30 min at room temperature**.
6. Remove adhesive foil and aspirate each well and **wash as step 4**.
7. Add **100 µl** of **TMB Substrate Reagent** to each well. Incubate for **10 minutes at RT in the dark** (without adhesive foil).
8. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough

mixing.

9. Read the OD with a microplate reader at **450 / 650 nm** immediately. It is recommended read the absorbance **within 30 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls or samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

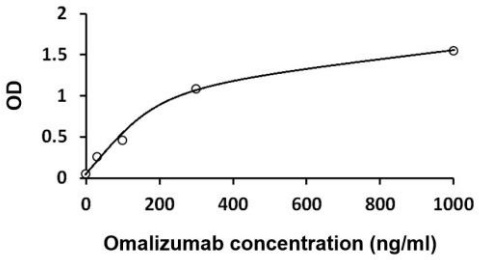
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. To obtain the exact values of the samples, the concentration determined from the standard curve must be multiplied by the dilution factor (200x). (Please refer the sample preparation in REAGENT PREPARATION section). If the samples have been further diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

This kit detects Omalizumab. The lowest detectable level that can be distinguished from the zero standard is 30 ng/mL.

Specificity

There is no cross reaction with native serum immunoglobulins and tested monoclonal antibodies such as infliximab (Remicade®), adalimumab (Humira®), etanercept (Enbrel®), bevacizumab (Avastin®) and trastuzumab (Herceptin®)

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay were < 15%.

Recovery

85-115% (normal human serum samples)