Anti-Omalizumab (Xolair®) ADA Qualitative ELISA

SKU: HUMB00040

Enzyme immunoassay for the qualitative determination (screening) of Omalizumab (Xolair®) in serum and plasma

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Required Volume (µl)	10
Total Time (min)	140
Sample	Serum, plazma
Sample Number	96
Detection Limit (ng/mL)	+/-
Spike Recovery (%)	-
Shelf Life (year)	1

Intended Use

The Elisa Genie Antibody to omalizumab (Xolair®) Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the qualitative determination of antibodies to omalizumab (Xolair®) in serum and plasma. It is for professional use only.

Summary and Explanation

A recombinant DNA-derived humanized IgG1k monoclonal antibody that selectively binds to human immunoglobulin E (IgE). Xolair is produced by a Chinese hamster ovary cell suspension culture in a nutrient medium containing the antibiotic gentamicin.

Xolair inhibits the binding of IgE to the high-affinity IgE receptor (FceRI) on the surface of mast cells and basophils. Reduction in surface-bound IgE on FceRI-bearing cells limits the degree of release of mediators of the allergic response. Xolair is used to treat severe, persisten asthma.

Xolair binds to IgE (a class of antibodies normally secreted in allergic responses), which prevents their binding to mast cells and basophils.

Most likely removed by opsonization via the reticuloendothelial system. Liver elimination of IgG includes degradation in the liver reticuloendothelial system (RES) and endothelial cells. Intact IgG is also excreted in bile.

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Test Principle

The Elisa Genie Antibody to omalizumab (Xolair®) ELISA is a sandwich assay for the determination of antibodies against omalizumab in serum and plasma samples. During the first incubation period, antibodies to omalizumab (AT0) in patient serum/ plasma samples are captured by the drug omalizumab (Xolair®) coated on the wall of the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled specific conjugate is added to each well and then incubated. After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The intensity of the reaction colour is directly proportional to the concentration of ATO in sample.

Warnings and Precautions

1. For professional use only.

2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.

3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.

6. Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.

7. Avoid contact with Stop solution. It may cause skin irritations and burns.

8. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.

9. All reagents of this test kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV by FDA approved procedures. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

Storage and Stability

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The strips of microtiter plate is stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2– 8°C.

Specimen Collection and Storage
Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles
Stability:	7 d	6 mon	

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

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with omalizumab.
1 x 0.25 mL	RCTV CNTR	**Reactive Control** Ready-to-use. Contains omalizumab -reactive reagent, human serum, stabilizers and <0.1% NaN_3
1 x 0.5 mL	NEG CNTR	**Negative Control** Ready-to-use. Contains human serum, stabilizers and <0.1% NaN_3
1 x 12 mL	ASSAY BUF	**Assay Buffer** Blue coloured. Ready to use. Contains proteins and <0.1% NaN3
1 x 12 mL	POD CONJ	**Peroxidase Conjugate** Red coloured. Ready to use. Contains peroxidase (POD) conjugate, stabilizer and preservatives.
1 x 12 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains TMB
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	**Wash Buffer, Concentrate (20x)** Contains Buffer with Tween 20.
2 x 1	ADH FILM	**Adhesive Film** For covering of Microtiter Plate during incubation.

Materials Required but not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Calibrated measures.
3. Tubes (1 mL) for sample dilution.
4. Wash bottle, automated or semi-automated microtiter plate washing system.
5. Microtiter plate reader capable of reading absorbance at 450/650 nm.
6. ddH_2O or deionised water, paper towels, pipette tips and timer.

Procedure Notes

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps must be performed strictly according to the instructions. Use calibrated pipettes and devices only.

2. 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.

3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.

4. Use a pipetting scheme to verify an appropriate plate layout.

5. 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.

6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.

7. 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.

Preparation of Components

Dilute/ dissolve	Component	with	Diluent	Rela-tion	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	ddH_2O	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	2 w

*. Prepare Wash Buffer before starting assay procedure.

Test Procedure

1	Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used.
2	**QUALITATIVE ELISA TEST FORMAT** Pipette 10 µL of ready-to use Negative Control, Reactive Control, and Samples into the respective wells of microtiter plate. <u>Wells</u> A1: Negative Control B1: Negative Control C1: Reactive Control D1 and on: Sample (Serum/Plasma)
3	Cover the plate with adhesive film. Briefly mix contents by gently shaking the plate. Incubate 60 min at room temperature (18-25°C).
4	Remove adhesive film. 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	Pipette 100 µL of ready-to use Peroxidase Conjugate into each well
6	Cover the plate with adhesive film. Incubate 60 min at room temperature (18- 25°C).
7	Remove adhesive film. 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.
8	Pipette 100 µL of TMB Substrate Solution into each well.
9	Incubate 20 min (without adhesive foil.) at room temperature (18-25°C) in the dark.
10	Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Briefly mix contents by gently shaking the plate. Colour changes from blue to yellow
11	Measure optical density with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution.

Interpretation of Results

For the run to be valid, the $OD_{450/650}$ nm of Positive Control should be ≥ 1.00 and the $OD_{450/650}$ nm of each Negative Control should be <0.200 0, 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.

E.g.;

If "Sample $OD_{450/650}$ /the mean $OD_{450/650}$ of Negative Controls" is ≥3, the sample is POSITIVE

If "Sample $OD_{450/650}$/the mean $OD_{450/650}$ of Negative Controls" is <3, the sample is NEGATIVE