MATRIKS BIOTEK
innovation for health & wellness

"trace & catch"

Instructions for Use

Omalizumab (Xolair®) ELISA

SHIKARI®

Q-OMA

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

REF **TR-OMAv1** Σ 12 x 8 2-8°C

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CE
IVD

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	SHIKARI® Q-OMA
	Free Omalizumab (Xolair®) quantitative analyses
Required Volume (µl)	5
Total Time (min)	70
Sample	Serum, plasma
Sample Number	96
Detection Limit (ng/mL)	10
Spike Recovery (%)	Between 85-115
Shelf Life (year)	1

Intended Use

Enzyme immunoassay for the quantitative determination of omalizumab (Xolair®) in serum and plasma. *Matriks Biotek® omalizumab ELISA* has been especially developed for the quantitative analysis omalizumab in serum and plasma samples between the Cmin and Cmax range of concentrations indicated in the pharmacokinetics section of prospectus.

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

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtitre plate coated with the reactant specific for omalizumab (Xolair®). Following incubation wells are washed and then horse radish peroxidase (HRP) conjugate is added and binds to omalizumab. After incubation, the wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. The color developed is proportional to the amount of omalizumab (Xolair®) in the sample or standard. Results of samples can be determined directly using the standard curve.

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 please refer to the local distributor.
3. In case of severe damage of the kit package please contact Matriks Biotek or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.

9. All reagents of this kit containing human serum (i.e. standards) have been tested and were found negative for HIV I/II, HBsAg and HCV. 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.

10. 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.

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 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:	2 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. 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®).

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant for detection of Omalizumab (Xolair®).
8 x 1 mL	STND A-F HIGH CNTRL LOW CNTRL	**Omalizumab Standards A-F, High Level Control, Low Level Control** 1000; 300; 100; 30; 10 and 0 ng/mL Ready to use. Used for construction of the standard curve. Contains omalizumab (Xolair®), human serum, stabilizer and <0.1% NaN_3.
1 x 50 mL	ASSAY BUF	**Assay Buffer** Blue colored. Ready to use. Contains proteins and <0.1% NaN_3.
1 x 12 mL	HRP CONJ	**Peroxidase Conjugate** Red colored. Ready to use. Contains horse radish peroxidase (HRP) and stabilizers.
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.
2x1	SLGP	**Semi-Log Graph Paper** For constructing standard curve and calculation of results

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. Bidistilled 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 pretreatment steps have to 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.

Pre-Test Setup Instructions

1. Preparation of Components

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

***.** Prepare Wash Buffer before starting assay procedure.

2.Dilution of Samples

Sample	To be diluted	With	Relation	Remarks
Serum/ Plasma	Initially 1:200	Assay Buffer	1:200	For dilution at 1:200; 5µl Sample + 995µl Assay Buffer

Patient samples with a concentration of infliximab above the measuring range are to be rated as > "Highest Standard (Standard A)". The result must not be extrapolated. The patient sample in question should be further diluted with Assay Buffer and retested.

Test Procedure

1	Pipette **100µl** of **Assay Buffer** non-exceptionally into each of the wells to be used.
2	Pipette **10 µL** of each **ready-to use Standards, High level control, Low level control and Diluted Samples** into the respective wells of microtiter plate. **Wells** A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1: Standard F G1: High Level Control Low H1: Level Control A2 and on: Sample (Serum/Plasma)
3	Cover the plate with adhesive foil. Incubate 30 min at room temperature (18-25°C).
4	Remove adhesive foil. 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 into each well.
6	Cover the plate with adhesive foil. Incubate 30 min at room temperature (18-25°C).
7	Remove adhesive foil. 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 10 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. Color 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.

Quality Control

The test results are only valid only if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards must be found within the acceptable ranges as stated above and/or label. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation & Interpretation of Results

1. Using the standards (1000; 300; 100; 30; 10; 0 ng/mL) disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each of 4 standards on the vertical (Y-axis) axis versus the corresponding omalizumab concentration on the horizontal (X-axis) axis, thus creating a standard curve by 4 points obtained.

2. The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of omalizumab from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the omalizumab concentration for the unknown sample.

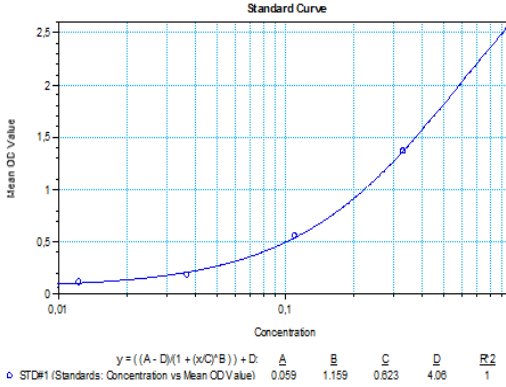
3. If computer data regation is going to be used, we recommend primarily "4 Parameter Logistic (4PL)" or secondly the "point-to-point calculation".

4. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (200x). Any sample reading greater than the highest standard should be further diluted appropriately with Assay Buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determind from the standard curve must be multiplied by the further dilution factor.

   E.g.; *If the pre-diluted sample further diluted in a ratio of 1:5 then results should be multiplied by 1000.*

5. Automated method: Computer programs can also generally give a good fit.

Typical Calibration Curve
(Example. Do not use for calculation!)



Standard	Concentration (ng/mL)	Mean OD 450/650
A	1000	2.595
B	300	1.361
C	100	0.550
D	30	0.182
E	10	0.115
F	0	0.054

Assay Characteristics

1. **Specificity:** There is no cross reaction with native serum immunoglobulins and tested monoclonal antibodies such as infliximab (Remicade®)Σ adalimumab (Humira®) , etanercept (Enbrel®), bevacizumab (Avastin®) ŀyŘ trastuzumab (Herceptin®)◊

2. **Sensitivity:** The lowest detectable level that can be distinguished from the zero standard is 10 ng/mL.

3. **Precision Of Kit:**
   Intra-assay CV: <15% for omalizumab range 1000-10 ng/mL
   Inter-assay CV: <15% for omalizumab range 1000-10 ng/mL

4. **Recovery:** Recovery rate was found to be between 85-115% with normal human serum samples with known concentrations.

Automation

Experiments have shown that the ***Matriks Biotek®*** **SHIKARI® Omalizumab ELISA** is also suitable to run on an automated ELISA processor.

References

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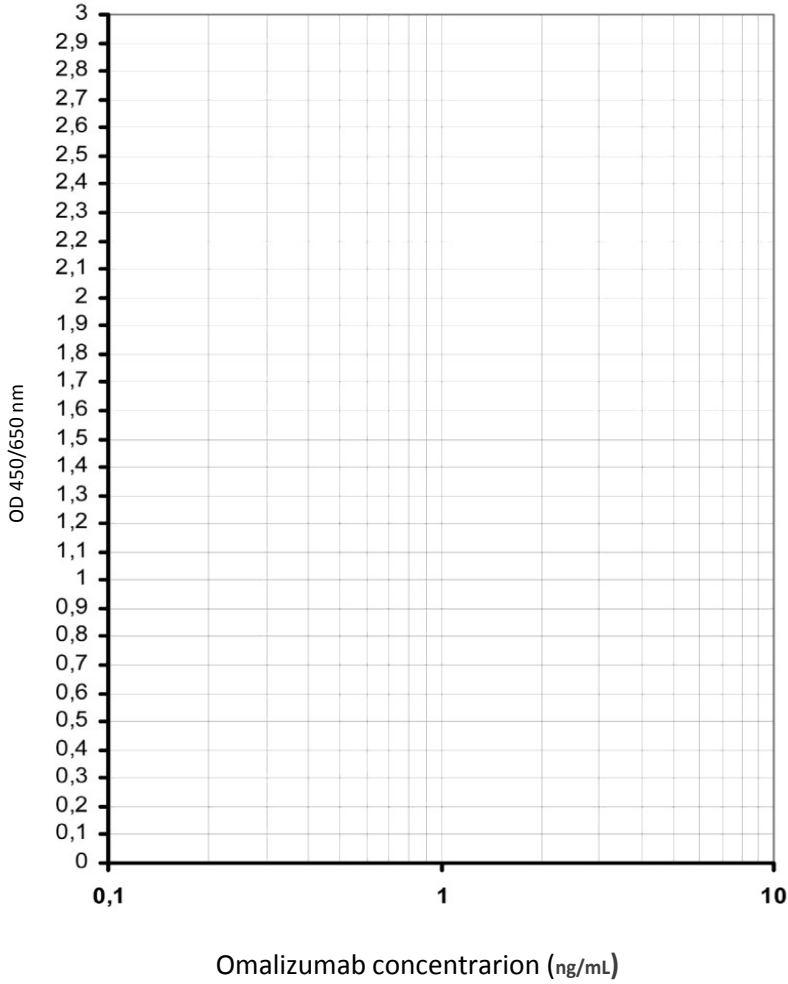
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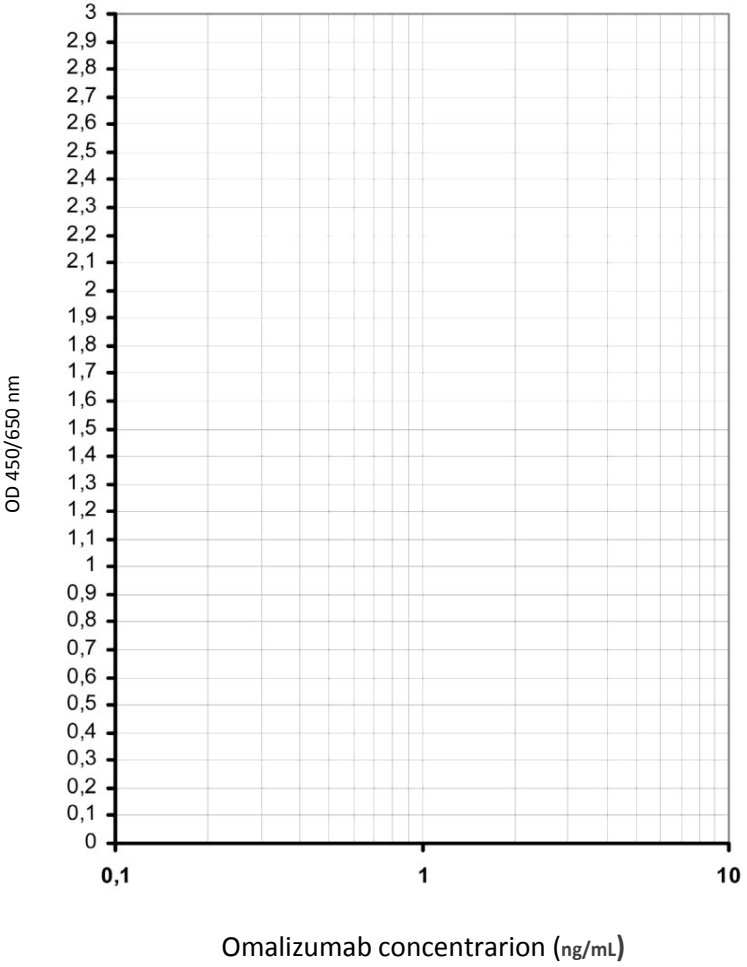
Omalizumab concentrarion (ng/mL)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Omalizumab concentrarion (ng/mL)