Instructions for Use

Omalizumab ELISA

Enzyme immunoassay for the measurement of free Omalizumab in human serum and plasma

REF: IG-AA111

Σ 12X8

For Research Use Only - Not for Use in Diagnostic Procedures

Distributed by
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1. INTENDED USE
The Eagle Biosciences Omalizumab ELISA Assay Kit is an enzyme immunoassay for the measurement of free Omalizumab in human serum and plasma samples. The Omalizumab ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

2. SUMMARY AND EXPLANATION
The drug Omalizumab (trade name Xolair®) is a recombinant DNA-derived humanized IgG1κ monoclonal antibody and it binds to human immunoglobulin E (IgE). The molecular weight of Omalizumab is 149 kilodaltons and is produced by a Chinese hamster ovary cell suspension culture. Omalizumab inhibits the binding of IgE to IgE receptor (FcεRI) on the surface of mast cells and basophils. Therefore, the Omalizumab is expected to limit the degree of release of mediators of the allergic response from the FcεRI bearing cells.

Identification of biomarkers for (non-)response and risk factors for adverse drug reactions that might be related to serum concentrations and maintaining the effective concentration of Omalizumab in order to potentially avoid some side effects with a reliable method might be beneficial.

3. PRINCIPLE OF THE TEST
The ImmunoGuide Omalizumab ELISA is a sandwich-type ELISA. Standards and diluted samples (serum or plasma) are incubated in the microtiter plate coated with human IgE. After incubation, the wells are washed. A horseradish peroxidase (HRP)-conjugated anti-human IgG monoclonal antibody is added and binds to the Fc part of Omalizumab pre-captured by the human IgE coated on the surface of the wells. Following incubation, the wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. The colour developed is proportional to the amount of Omalizumab in the sample or standard. Results of samples are determined directly by using the standard curve.

4. WARNINGS AND PRECAUTIONS
1. 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.
2. In case of severe damage of the kit package, please contact Eagle Biosciences or your supplier in writing, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
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.
Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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 have to 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. If any component of this kit contains human serum or plasma it is indicated and if so, it has been tested and were found to be negative for HIV I/II, HBsAg and HCV. However, the 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.
9. Some reagents contain sodium azide (NaN₃) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN₃ 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.

5. STORAGE AND STABILITY OF THE KIT

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 microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION, HANDLING 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.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Stability</th>
<th>2-8°C</th>
<th>≤-20°C (Aliquots)</th>
<th>Keep away from heat or direct sun light</th>
<th>Avoid repeated freeze-thaw cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 d</td>
<td>6 mon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Omalizumab ELISA Assay Kit
Catalog Number: IG-AA111

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7. CONTENTS OF THE KIT

<table>
<thead>
<tr>
<th>QUANTITY</th>
<th>COMPONENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 12 x 8</td>
<td>Microtiter Plate</td>
</tr>
<tr>
<td></td>
<td>Break apart strips coated with human IgE</td>
</tr>
<tr>
<td>5 x 2 mL</td>
<td>Omalizumab Standards A-E</td>
</tr>
<tr>
<td></td>
<td>300; 100; 30; 10; and 0 ng/mL</td>
</tr>
<tr>
<td></td>
<td>Ready to use. Used for construction of the standard curve. Contains Omalizumab, proteins, stabilizer and &lt;15mM NaN₃.</td>
</tr>
<tr>
<td>2 x 60 mL</td>
<td>Assay Buffer</td>
</tr>
<tr>
<td></td>
<td>Blue colored. Ready to use. Contains proteins and &lt;15mM NaN₃.</td>
</tr>
<tr>
<td>1 x 12 mL</td>
<td>Enzyme Conjugate</td>
</tr>
<tr>
<td>1 x 12 mL</td>
<td>TMB Substrate Solution</td>
</tr>
<tr>
<td></td>
<td>Ready to use. Contains 3,3’,5,5’-Tetramethylbenzidine (TMB).</td>
</tr>
<tr>
<td>1 x 12 mL</td>
<td>Stop Solution</td>
</tr>
<tr>
<td></td>
<td>Ready to use. 1 N Hydrochloric acid (HCl).</td>
</tr>
</tbody>
</table>
| 1 x 50 mL | Wash Buffer, Concentrate (20x)
|           | Contains buffer, Tween® 20 and Kathon™. |
| 2 x 1     | Adhesive Seal |
|           | For sealing microtiter plate during incubation. |

8. MATERIALS REQUIRED BUT NOT SUPPLIED
1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Bidistilled or deionized water and calibrated glasswares (e.g. flasks or cylinders).
3. Wash bottle, automated or semi-automated microtiter plate washing system.
4. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength at 600-650 nm is optional).
5. Absorbent paper towels, standard laboratory glass or plastic vials, and a timer.

9. 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 readily at the appropriate time. Allow all reagents and specimens to

12.1. SPECIFICITY
There is no measurable cross reaction with other therapeutic antibodies (Following drugs were tested at 50µg/mL: Infliximab, Adalimumab, Golimumab, Etanercept, Trastuzumab, Rituximab, Bevacizumab and Tocilizumab) and native serum immunoglobulins. Experiments have demonstrated that binding of Omalizumab to the microtiter plate is inhibited if added into the wells simultaneously with IgE.

12.2. SENSITIVITY
The lowest detectable level that can be clearly distinguished from the zero standard is less than 1 ng/mL (zero standard +2SD read from the curve).

12.3. PRECISION OF THE KIT
Intra-assay CV: <10%.
Inter-assay CV: <10%.

12.4. RECOVERY
Recovery rate was found to be 97-105% with native human serum (n=8) of unknown IgE contents. In addition, three different human serum with total IgE of 101 IU/mL, 200 IU/mL, and 85.5 IU/mL were also spiked with exogenous Omalizumab at 15 µg/mL and then recovery rates were measured as 98%, 97% and 97% respectively.

13. AUTOMATION
Experiments have shown that the ImmunoGuide Omalizumab ELISA is suitable also for using by an automated ELISA processor.

14. REFERENCES
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange the caps of vials. Always cap not used vials. Do not reuse wells 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 Micropipettor 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. Do not open the pouch until it reaches room temperature. Unused wells should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Components*

<table>
<thead>
<tr>
<th>Diluted/dissolve</th>
<th>Component</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL</td>
<td>Wash Buffer</td>
<td>up to 200mL</td>
<td>1:20</td>
<td>Warm up at 37°C to dissolve crystals. Mix vigorously.</td>
<td>2.8 °C</td>
<td>4 w</td>
</tr>
</tbody>
</table>

* Prepare Wash Buffer before starting the assay procedure.

10.2. Dilution of Samples*

<table>
<thead>
<tr>
<th>Sample</th>
<th>To be diluted</th>
<th>With</th>
<th>Relation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/ Plasma</td>
<td>Initially 1:200</td>
<td>Assay Buffer</td>
<td>1:200-1:1000</td>
<td>For dilution at 1:200; 5µl Sample + 995µl Assay Buffer For dilution at 1:500; 50µl of 1:200 diluted Sample + 200µl Assay Buffer</td>
</tr>
</tbody>
</table>

* If any sample, initially diluted as indicated above, produces an OD value above the measuring range it should be rated as “> highest standard”. The result should not be extrapolated. The sample in question should be further diluted with Assay Buffer and then retested.

11. TEST PROCEDURE

11.1. GENERAL REMARKS
11.1.1. Before performing the assay, samples and assay kit should be brought to room temperature (about 30 minutes beforehand) and ensure the homogeneity of the solution.
11.1.2. All Standards should be run with each series of unknown samples.
11.1.3. Standards should be subject to the same manipulations and incubation times as the samples being tested.
11.1.4. All steps of the test should be completed without interruption.
11.1.5. Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.

11.2. ASSAY PROCEDURE

1. Pipette 100 µL of each Ready-to Use Standard, and Diluted Samples into the respective wells of the microtiter plate.

<table>
<thead>
<tr>
<th>Wells</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1: Standard A</td>
<td>300</td>
</tr>
<tr>
<td>B1: Standard B</td>
<td>100</td>
</tr>
<tr>
<td>C1: Standard C</td>
<td>30</td>
</tr>
<tr>
<td>D1: Standard D</td>
<td>10</td>
</tr>
<tr>
<td>E1: Standard E</td>
<td>0</td>
</tr>
<tr>
<td>F1 and so on: Diluted samples (Serum/Plasma)</td>
<td></td>
</tr>
</tbody>
</table>

2. Cover the plate with adhesive seal. Shake plate carefully. **Incubate 60 min at room temperature (RT) (20-25°C).**

3. Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate 3 X 300 µL of Diluted Wash Buffer per well. Remove excess solution by tapping the inverted plate on a paper towel.

4. Pipette 100 µL of Enzyme Conjugate (HRP-anti human IgG mAb) into each well.

5. Cover plate with adhesive seal. Shake plate carefully. **Incubate 30 min at RT.**

6. Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate 3 X 300 µL of Diluted Wash Buffer per well. Remove excess solution by tapping the inverted plate on a paper towel.

7. Pipette 100 µL of Ready-to-Use TMB Substrate Solution into each well.

8. Incubate 15 min at RT. Avoid direct sunlight.

9. 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.

10. Measure optical density (OD) with a photometer at 450 nm (Reference at OD630 nm is optional) within 15 min after pipetting the Stop Solution.

11.3. QUALITY CONTROL

The test results are only valid 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/controls 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 reviewed: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

11.4. CALCULATION OF RESULTS

A standard curve should be calculated using the standard concentration (X-axis) versus the OD450 (or OD450/620) values (Y-axis). This can be done manually using graph paper or with a computer program. Concerning the data regression by computer we are recommending to primarily use the "4 Parameter Logistic (4PL)" or alternatively the "point-to-point calculation". In case of manual plot there are 2 options: Semilog graph (see Fig. A) or linear graph (see Fig. B). Semilog graph paper is available at [http://www.papersnake.com/logarithmic/semilogarithmic/](http://www.papersnake.com/logarithmic/semilogarithmic/).

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 the drug from the standard curve. This value always has to be multiplied by the dilution factor. If any diluted sample is reading greater than the highest standard, it should be further diluted appropriately with Assay Buffer and retested. Also this second dilution has to be used for calculation the final result.

Typical Calibration Curve

(Just an example. Performed at 23°C. Do not use it for calculation!)

<table>
<thead>
<tr>
<th>Standard</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (ng/mL)</td>
<td>300</td>
<td>100</td>
<td>30</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Mean OD450/620nm</td>
<td>1.750</td>
<td>1.280</td>
<td>0.811</td>
<td>0.414</td>
<td>0.023</td>
</tr>
</tbody>
</table>

**Fig. A**

![Graph A](http://example.com/graphA.png)

**Fig. B**

![Graph B](http://example.com/graphB.png)

12. ASSAY CHARACTERISTICS

Omalizumab (ng/mL)