



## User's Manual

# Tocilizumab ELISA Kit



DEIASL021



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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

Enzyme immunoassay for determination of quantitative antibodies to Tocilizumab in serum and plasma samples

### General Description

Tocilizumab, also known as atlizumab, is an immunosuppressive drug, mainly for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis, a severe form of arthritis in children. It is a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R). Interleukin 6 (IL-6) is a cytokine that plays an important role in immune response and is implicated in the pathogenesis of many diseases, such as autoimmune diseases, multiple myeloma and prostate cancer. It was developed by Hoffmann–La Roche and Chugai.

### Principles of Testing

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtiter plate coated with the reactant for tocilizumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to tocilizumab captured by the reactant on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of

tetramethylbenzidine (TMB) chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed is proportional to the amount of tocilizumab in the sample or standard. Results of samples can be determined directly using the standard curve.

### Reagents And Materials Provided

1. Microtiter Plate: 1 × 12 × 8, Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant.
2. Standard A-E: 0.3 mL (each)  
Standard A: 3000 ng/mL  
Standard B: 1000 ng/mL  
Standard C: 300 ng/mL  
Standard D: 100 ng/mL  
Standard E: 0 ng/mL  
  
Ready to use. Used for the standard curve and control. Contains tocilizumab, human serum and stabilizer, < 0,1 % NaN<sub>3</sub>.
3. Control low and high levels: 0.3 mL (each) Ready to use. Contains human serum and stabilizer, < 0,1 % NaN<sub>3</sub> Control concentrations are given in COA.
4. Assay Buffer: 2 × 50 mL, Ready to use. Blue coloured. Contains proteins, < 0,1 % NaN<sub>3</sub>.

5. Conjugate: 1 × 12 mL, Horse radish peroxidase. Conjugated probe ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.
6. Substrate: 1 × 12 mL, TMB substrate solution. Ready to use. Contains 3,3',5,5'- Tetramethylbenzidine (TMB).
7. Stop Buffer: 1 × 12 mL, Ready to use. 1N HCl.
8. Wash Buffer (20×): 1 × 50 mL, Prepared concentrated (20×) and should be diluted with the dilution rate given in the "Reagent Preparation" before the test. Contains buffer with tween 20.
9. Foil: 2 × 1, For covering microtiter plate during incubation.

## Materials Required But Not Supplied

1. Micropipettes and tips
2. Calibrated measures
3. Tubes for sample dilution
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of measuring optical density with a photometer at OD 450 nm with reference wavelength 650 nm (450/650 nm)
6. Distilled or deionised water, paper towels, pipette tips and timer

## Storage

The kit is shipped at ambient temperature (10-30°C) and should be stored at 2-8°C for long term storage. Keep away from heat or direct sunlight. The strips of microtiter plate are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

## Reagent Preparation

### 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 eight-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.

- 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.

**Component:**

**Wash buffer** (Must be prepared before starting assay procedure)

Dilute: 10 mL (e.g.)

With Distilled water: Up to 200 mL

Dilution Ratio: 1/20

Remarks: Warm up 37°C to dissolve crystals. Mix vigorously

Storage: 2-8°C

Stability: 2 weeks

**Dilution of Standards and controls**

Sample: Standards and controls

Diluent: Assay buffer

Dilution Ratio: 1/10

Remarks: For final dilution 1/10, 20 µL sample + 180 µL assay buffer

**Dilution of samples**

Sample: Serum/Plasma

Diluent: Assay buffer

Dilution Ratio: 1/1000

Remarks: For final dilution 1/1000,

– First step: 1/10 dilution  
10 µL sample + 90 µL assay buffer

– Second step: 1/100 dilution  
5 µL sample (1/10 diluted) + 495 µL assay buffer

Patient samples with a concentration of drug 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.

**Assay Procedure**

Total assay time: 70 minutes

- Dilute each of the standards, controls and samples as described in "Reagent Preparation"

2. Pipette 100 µL of each diluted "Standards", "Low level control", "High level control" and 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: Low level control

G1: High level control

3.
  - Cover the plate with adhesive foil
  - Briefly mix contents by gently shaking the plate
  - Incubate 60 minutes at room temperature (18-25°C)
4.
  - Remove adhesive foil
  - Discard incubation solution
  - Wash plate three times each with 300 µL "Wash Buffer"
  - Remove excess solution by tapping the inverted plate on a paper towel
5. Pipette 100 µL "Conjugate" into each well
6.
  - Cover the plate with adhesive foil
  - Incubate 30 minutes at room temperature (18-25°C)
7.
  - Remove adhesive foil
  - Discard incubation solution
  - Wash plate three times each with 300 µL "Wash Buffer"
  - Remove excess solution by tapping the inverted plate on paper towel
8.
  - Pipette 100 µL "Substrate" into each well
9.
  - Incubate 10 minutes without adhesive foil at room temperature (18-25°C) in the dark
10.
  - Stop the substrate reaction by adding 100 µL "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 OD 450 nm with reference wavelength 650 nm (450/650 nm) within 30 minutes after pipetting the "Stop Solution"

**The ELISA Kit is also suitable to run on automated ELISA processors.**

## 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. For a valid study, the OD 450/650 of the highest standard should be >1,500 and the OD 450/650 of the lowest



standard should be  $<0,150$  in case of any deviation the following technical issues (but not limited to) should be reviewed: Expiration dates of reagents, storage conditions, pipettes, devices, incubation conditions, washing methods, etc.

## Calculation

1. Create a standard curve by using the standards. OD 450/650 nm for each standard on the vertical (Y-axis) axis versus the corresponding drug concentration on the horizontal (X-axis) axis.
2. Standards and controls are prepared concentrated (10 $\times$ ). They should be diluted with the dilution rate given in the "Reagent Preparation" before the test. The standard curve should be prepared with the values obtained after dilution.
3. 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 drug 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 drug concentration of the unknown sample.
4. If computer data is going to be used, we recommend primarily "Four Parameter Logistic (4PL)" or secondly the "point-to-point calculation".
5. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (1000 $\times$ ). 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 determined 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 5000.

6. For low and high level controls values, refer to "Quality Control Certificate" provided by each kit.

## Precision

Intra-assay and inter-assay CVs < 30%

## Sensitivity

The lowest detectable level (Lowest detection limit, LOD) that can be distinguished from the zero standard is 0,003  $\mu\text{g/ml}$

Functional sensitivity (Limit of quantification-LOQ): 0,01  $\mu\text{g/mL}$

## Specificity

The kit is shipped at ambient temperature (10-30°C) and should be stored at 2-8°C for long term storage. Keep away from heat or direct sunlight. The strips of microtiter plate are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

## Recovery

< 100 ± 30%

## Precautions

1. For professional use only.
2. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
3. 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.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. All reagents of this kit containing human serum or plasma (standards etc.) have been tested and were found negative for HIV I/II, HBsAg and Anti-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.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations.
7. Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.

