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Datasheet for ABIN2862658
Infliximab Antibody ELISA Kit

3 Images

Overview

Quantity:	96 tests
Target:	Infliximab Antibody
Binding Specificity:	Free Chain
Reactivity:	Chemical, Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Enzyme immunoassay for the semi-quantitative determination of free antibodies to Infliximab (ATI) in human serum and plasma.
Brand:	ImmunoGuide®
Sample Type:	Serum, Plasma (EDTA - heparin)
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	Free antibodies against Infliximab (Remicade®, Remsima®) Because of our special assay design this Antibody to Infliximab ELISA kit provides the advantage of limiting the potential false positive reactions that are related with the presence of RF in serum/plasma samples. A screening test was performed with 56 different native human sera. All samples showed OD450 nm values (ranged from 0.053 to 0.083) less than the mean OD of cut-off controls. This test system measures the concentration of free antibodies directed against Infliximab. It cannot detect these antibodies if the drug already is bound to it.

Product Details

Sensitivity:	10 ng/mL
Characteristics:	This test does not measure the antibodies if they already are bound to the drug Infliximab.
Components:	<ul style="list-style-type: none">• 1 x 12 x 8 Microtiter Plate Break apart strips pre-coated with the drug Infliximab.• 1 x 0.5 mL Negative Control Ready to use. Contains human serum and <15 mM NaN₃.• 1 x 0.5 mL Cut-off Control Ready to use. Contains human serum antibody to Infliximab at 3 AU/mL and <15 mM NaN₃.• 1 x 0.5 mL Positive Control Ready to use. Contains human serum with Antibody to Infliximab and <15 mM NaN₃.• 1 x 12 mL Assay Buffer Blue colored. Ready to use. Contains proteins and <15mM NaN₃.• 1 x 12 mL Enzyme Conjugate Red colored. Ready to use. Contains horseradish peroxidase(HRP)- conjugated Infliximab, Proclin® and stabilizers.• 1 x 12 mL TMB Substrate Solution Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB).• 1 x 12 mL Stop Solution Ready to use. 1 N Hydrochloric acid (HCl).• 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.
Material not included:	<ul style="list-style-type: none">• Micropipettes (< 3 % CV) and tips to deliver 5-1000 µL.• Bidistilled or deionised water and calibrated glasswares (e.g. flasks or cylinders).• Wash bottle, automated or semi-automated microtiter plate washing system.• Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength at 600-650 nm is optional).• Absorbent paper towels, standard laboratory glass or plastic vials, and a timer.

Target Details

Target:	Infliximab Antibody
Target Type:	Antibody
Background:	<p>Infliximab is a chimeric monoclonal antibody and used to treat auto-immune disorders.</p> <p>Infliximab reduces the amount of active human tumour necrosis factor alpha (hTNFα) in the patient by binding to it and preventing it from signaling the receptors for TNFα on the surface of various cell types. TNFα is one of the key cytokines that triggers and sustains the inflammatory reactions. Infliximab is used for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, ulcerative colitis. This drug is approved by FDA. One of the major concern, despite of its wide usage, is the potential development of anti-Infliximab antibodies (ATI) which in turn may interfere with Infliximab efficacy as mainly judged by observing the relapse of signs and symptoms of disease and necessitate dose-escalation or potentially ending up the treatment. In this context, demonstration of anti-Infliximab antibodies</p>

Target Details

during treatment with Infliximab has a major concern and monitoring for the presence of specific antibodies during clinical trials is an important issue for follow up of the treatment regimens. The Antibody to Infliximab ELISA Kit can be efficiently used for monitoring Infliximab-specific antibodies during therapy and offers the clinician a tool for decision on possible preventive measures such as possible addition of immunosuppressive drug(s) to reduce anti-Infliximab antibodies.

Molecular Weight: 144 kDa

Application Details

Application Notes:

- 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.
- All Standards should be run with each series of unknown samples.
- Standards should be subject to the same manipulations and incubation times as the samples being tested.
- All steps of the test should be completed without interruption.
- Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.

Comment: Antibody to Infliximab ELISA is suitable also for using by an automated ELISA processor.

Sample Volume: 10 μ L

Assay Time: 2.5 h

Plate: Pre-coated

Protocol: The Antibody to Infliximab ELISA is a sandwich type ELISA for the determination of antibodies against Infliximab in serum and plasma samples. During the first incubation period, antibodies to Infliximab (ATI) in patient serum/plasma samples are captured by the drug Infliximab coated on the wall of the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled Infliximab conjugate is added and then incubated. ATI, if present in sample, will make a bridge, with its two identical Fab arms, between the Infliximab coated on the well and the other Infliximab labelled with peroxidase. After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated with stop solution. The positive reaction is related with the presence of ATI in the sample.

Reagent Preparation: Wash Buffer: Dilute 10 mL Wash Buffer (up to 200 mL) at the ratio of 1:20 with distilled water.
Warm up at 37 °C to dissolve crystals. Mix vigorously.
Store at 2-8 °C for up to 4 weeks.

Application Details

Prepare Wash Buffer before starting the assay procedure.

Sample Collection: Normal serum or plasma collection

Sample Preparation: 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 &leq,-20 °C (Aliquots)

Keep away from heat or direct sun light.

Avoid repeated freeze-thaw cycles.

Stability: 3 days at 2-8 °C, 6 months at -20 °C

Assay Procedure:

1. Pipette 100 µL of Assay Buffer into each of the wells to be used.
2. Pipette 10 µL of each Ready-to Use Negative Control, Cut-off Control, Positive Control, and Samples into the respective wells of microtiter plate. Wells A1: Negative Control B1: Negative Control C1: Cut-off Control D1: Cut-off Control E1: Positive Control F1: Positive Control G1 and so on: Samples (Serum/Plasma)
3. Cover the plate with adhesive seal. Shake plate carefully. Incubate 60 min at room temperature (RT) (20-25 °C).
4. 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.
5. Pipette 100 µL of Enzyme Conjugate (HRP-Infliximab) into each well.
6. Cover plate with adhesive seal. Shake plate carefully. Incubate 60 min at RT.
7. 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.
8. Pipette 100 µL of Ready-to-Use TMB Substrate Solution into each well.
9. Incubate 15 min at RT. Avoid exposure to direct sunlight..
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 (OD) with a photometer at 450 nm (Reference at OD620 nm is optional) within 15 min after pipetting the Stop Solution.

Calculation of Results: For the run to be valid, the OD450 nm of each Positive Control should be ≥ 1.000 and the OD450 nm of each Negative Control should be ≤ 0.150 . If not, improper technique or reagent deterioration may be suspected and the run should be repeated. The results are evaluated by dividing all individual results by the mean OD450nm of the Cut-off Controls. The results are expressed in arbitrary units (AU/mL).

Application Details

Cut-off= 3 AU/mL=OD Cut-off Control

Samples which have an equal and higher optical density (OD) than the mean OD of cut-off controls are positive.

Range: ≥ 3 AU/mL (Positive)

Range: < 3 AU/mL (Negative)

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations.

Sample calculation for a positive sample:

OD of patient's sample = 0.800

Average OD of cut-off controls = 0.200 (3 AU/mL)

Concentration of patient's sample = $0.800/0.200 \times 3$ AU/mL = 12 AU/mL

Assay Precision:	Intra-assay CV: <10%. Inter-assay CV: <10%
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Restrictions:	For Research Use only
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Handling

Buffer:	< 15mM NaN3
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Preservative:	Sodium azide
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Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
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Storage:	4 °C
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Storage Comment:	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.
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Expiry Date:	24 months
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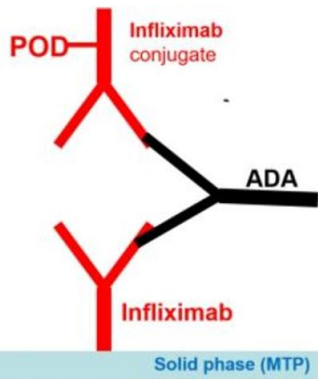


Image 1.

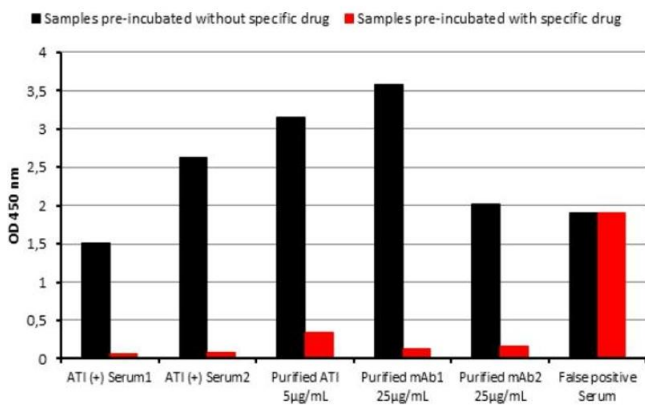


Image 2.

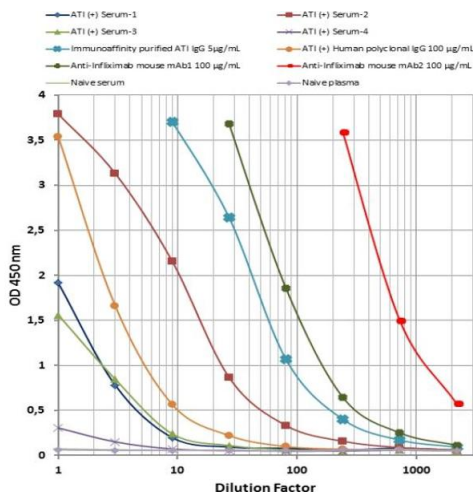


Image 3.