

ImmunoGuide[®]

Instructions for Use

Antibody to Infliximab (ATI) ELISA (Quantitative)

Enzyme immunoassay for the quantitative determination of antibodies to infliximab in human serum and plasma

REF IG1002-ATiv1

 12X8



2-8°C

For research use only

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1. INTENDED USE

The Immunoguide[®] Antibody to Infliximab (ATI) Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative determination of antibodies to Infliximab (Remicade[®])* in serum and plasma. The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations.

* Remicade[®] is a registered trademark of Centacor Ortho Biotech Inc.

2. SUMMARY AND EXPLANATION

Infliximab (Remicade[®]) is a chimeric monoclonal antibody and used to treat auto-immune disorders. Infliximab reduces the amount of active human tumour necrosis factor alpha (hTNF α) in the body by binding to it and preventing it from signaling the receptors for TNF α on the surface of various cell types. TNF α is one of the key cytokines that triggers and sustains the inflammatory reactions. Infliximab (Remicade[®]) is used for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, ulcerative colitis, and approved by FDA. One of the major concern, despite of its wide usage, is potential development of anti-infliximab antibodies (ATI) which in turn may interfere with infliximab (Remicade[®]) 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 ATI during treatment with infliximab has a major concern and monitoring for the presence and/or quantitation of specific antibodies during clinical trials is an important issue for follow up of the treatment regimens. The Immunoguide ATI ELISA Kit could be efficiently used for monitoring infliximab-specific antibodies (ATI) during therapy and offers the clinician a tool for decision on possible preventive measures to reduce anti-infliximab antibodies.

3. TEST PRINCIPLE

The Immunoguide Antibody to Infliximab (ATI) ELISA is a sandwich assay for the determination of antibodies against Infliximab in serum and plasma samples. During the first incubation period, 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, horse radish peroxidase (HRP)-conjugated infliximab 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 ATI in sample.

4. WARNINGS AND PRECAUTIONS

1. For research use only. 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. In case of severe damage of the kit package please contact Immunoguide 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. 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.
10. 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.

5. 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.

6. 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	

*. **Infliximab (Remicade®) infusion camouflages/masks the presence of antibody to infliximab (ATI) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATI. It's suggested to obtain blood sample just before the infusion of infliximab (Remicade®) or at least 2 weeks after the infusion of infliximab (Remicade®).**

7. MATERIALS SUPPLIED

1 x 12 x 8	MTP	Microtiter Plate Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with Infliximab.
5 x 0.3 mL	STND A-E	ATI Standards A-E 500; 250; 125; 62; 0 ng/mL Ready to use. Used for construction of the standard curve. Contains immunoaffinity-purified ATI, human serum, and <0.1% NaN ₃ .
1 x 50 mL	ASSAY BUF	Assay Buffer Blue colored. Ready to use. Contains proteins, RF blockers and <0.1% NaN ₃ .
1 x 12 mL	HRP CONJUG	Peroxidase Conjugate Red colored. Ready to use. Contains HRP-Conjugated Infliximab, 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	WASH BUF CONC	Wash Buffer, Concentrate (20X) Contains Buffer with Polysorbate 20.
2 x 1	ADHSV FILM	Adhesive Film For covering of Microtiter Plate during incubation.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette, < 3% CV).
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 nm.
6. Bidistilled or deionised water, paper towels, pipette tips and 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 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 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/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.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Components

Dilute/ Dissolve	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	4 w

*. Prepare Wash Buffer before starting assay procedure.

10.2. Dilution of Samples*

Sample	To be diluted	With	Relation	Remarks
Serum/Plasma	Initially no	Assay Buffer	1:10-1:100	For dilution at 1:10 10µl Sample+90µl Assay Buffer For dilution at 1:100: 5µl Sample+495µl Assay Buffer

*. Patient sample with a concentration of ATI producing an OD greater than the highest standard should be rated as "> highest standard". The result must not be extrapolated. The patient sample in question should be diluted with Assay Buffer and then retested.

11. TEST PROCEDURE

1.	Pipette 100 µL of Assay Buffer non-exceptionally into each of the wells to be used.
2.	Pipette 10 µL of each ready-to use Standards, and Samples into the respective wells of microtiter plate. <u>Wells</u> A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1: Standard F G1 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 the 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 film) at room temperature (18-25°C) in the dark.
10.	Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Color changes from blue to yellow. Briefly mix contents by gently shaking the plate.
11.	Measure optical density with a photometer at 450 nm within 30 min after pipetting of the Stop Solution.

12. 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 in the lot specific QC Sheet. 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.

13. INTERPRETATION & CALCULATION OF RESULTS

13.1. QUALITATIVE INTERPRETATION

13.1.1. The results are evaluated by a cut-off value which is estimated by multiplying the OD_{450nm} of the zero Standard by 3.

If "Sample OD₄₅₀/Zero Standard OD₄₅₀" is <3, the sample is NEGATIVE for ATI

If "Sample OD₄₅₀/Zero Standard OD₄₅₀" is ≥3, the sample is POSITIVE for ATI and should be extrapolated for quantitative analysis.

13.2. QUANTITATIVE INTERPRETATION

13.2.1. Using the standards (0, 62, 125, 250, 500 ng/mL), construct a standard curve by plotting the OD_{450 nm} for each of 5 standards on the vertical (Y-axis) axis versus the corresponding ATI concentration on the horizontal (X-axis) axis, thus creating a standard curve by 5 points obtained.

13.2.2. 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 ATI 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 ATI concentration for the unknown sample.

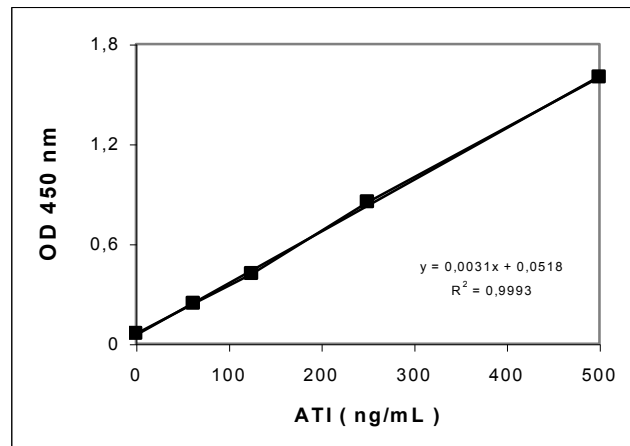
13.2.3. Any sample reading greater than the highest standard should be diluted appropriately with Assay Buffer and retested. Therefore, if the samples have been diluted, the concentration determined from the standard-curve must be multiplied by the dilution factor.

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

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	ATI (ng/mL)	Mean OD
A	500	1.600
B	250	0.850
C	125	0.420
D	62	0.240
E	0	0.060



14. ASSAY CHARACTERISTICS

14.1. SPECIFICITY

There is no cross reaction with any other proteins present in native human serum. Infliximab (Remicade®) infusion camouflages/masks the presence of antibody to infliximab (ATI) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATI. It is convenient to obtain blood sample just before the infusion of infliximab or at least 2 weeks after the infusion of infliximab.

14.2. SENSITIVITY

The lowest detectable level that can be distinguished from the zero standard is <30 ng/mL.

14.3. PRECISION OF KIT

Intra-assay CV: <9% for the ATI range of 62-500 ng/mL.

Inter-assay CV: <9% for the ATI range of 62-500 ng/mL.

14.4. RECOVERY

Recovery rate was found to be higher than 97% with native human sera spiked with infliximab at known concentrations.

15. AUTOMATION

Experiments have shown that the Immunoguide ATI ELISA is also suitable to run on an automated ELISA processor.

16. REFERENCES

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