ImmunoGuide®

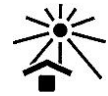
Instructions for Use

Total Antibody to Infliximab ELISA

Enzyme immunoassay for the semi-quantitative determination of total (free and drug-bound) antibodies to Infliximab in serum and plasma

REF: IG-TA101

12X8



2-8°C

CE

IVD

AYBAYTECH Biyoteknoloji Ltd. Sti.
Macun Mah. Batı Blv. ATB İş Merkezi No: 1/285,
06374-Yenimahalle, Turkey
Tel : +90 312 397 88 05
Fax : +90 312 397 88 06
e-mail: info@aybaytech.com
www.aybaytech.com

Version.-No.: TA101CEIVD
2020/1 CA

Contents **Page**

1. INTENDED USE

Enzyme immunoassay for the semi-quantitative determination of total (free and drug-bound) antibodies to Infliximab (ATI) in serum and plasma.

2. SUMMARY AND EXPLANATION

The drug Infliximab (trade name Remicade®, Remsima®, or Inflectra®) is a chimeric monoclonal antibody and used to treat auto-immune disorders. One of the major concerns, despite of its wide usage, is the potential development of anti-Infliximab antibodies (ATI) which in turn may interfere with the drug efficacy as mainly judged by observing the relapse of signs and symptoms of disease and necessitate dose-escalation or potentially ending up the treatment.

The ImmunoGuide Total Antibody to Infliximab ELISA Kit can be efficiently used for measuring free and Infliximab-bound antibodies against the drug Infliximab. In combination with the drug level determination, the *ImmunoGuide* Total Antibody to Infliximab ELISA Kit has been designed for the measurement of free antibodies against this drug. It also detects such antibodies which already are bound to the drug, even if the drug is present in a 20 fold excess.

3. PRINCIPLE OF THE TEST

The *ImmunoGuide* Antibody to Infliximab ELISA serves for the determination of total antibodies (free and Infliximab-bound) against Infliximab in serum and plasma samples. During sample preparation, the Infliximab-bound anti-Infliximab antibodies are dissociated from the complex in order to acquire free anti-Infliximab antibodies. During the incubation period, antibodies to Infliximab (ATI) are replaced and bridged in the well with its Fab arms, between the Infliximab coated on the well and the conjugate Infliximab labelled with peroxidase. After washing away the components 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.

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 ***AybayTech*** 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.
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 preservatives. In case of contact with eyes or skin, flush immediately with water.

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.

Storage:	2-8°C	≤-20°C (Aliquots)	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles
Stability:	3 d	6 mon	

***** Drug administration/infusion may camouflages/masks the presence of anti-drug antibody (ADA) in serum/plasma samples. Therefore, blood sampling time is also critical for detection of ADA. It is proposed to obtain blood sample just before administration of the drug.

7. CONTENTS OF THE KIT

QUANTITY	COMPONENT
1 x 12 x 8	**Microtiter Plate** Break apart strips pre-coated with the drug Infliximab.
1 x 1 mL	**Negative Control** Contains serum and preservative.
1 x 1 mL	**Positive Control** Contains Infliximab-specific antibody and preservative.
1 x 12 mL	**Dissociation Buffer** Ready to use. Contains buffer.
1 x 12 mL	**Assay Buffer** Ready to use. Contains buffer.
1 x 6 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™.
1 x 1	**Polypropylene Microtiter Plate** For the usage of sample and control dissociation
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 deionised 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 pre-treatment 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 reach room temperature (20-25 °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 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*

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
10 mL	Wash Buffer	up to 200 mL	Distilled Water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	4 w

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

10.2. Preparation of Samples and Controls

10.2.1. Mix the samples and controls (Negative and Positive) 1:5 in dissociation buffer by pipetting 20µL of sample and controls separately into a reaction tube or into the wells of an empty polypropylene microtiter plate included (NOT the drug-coated polystyrene ELISA plate also included in this Kit) and adding 80µl dissociation buffer. Mix well by gently tapping to make turbulence or flushing up and down 3-5 times using a micropipette. Carry out this step without interruption since this step dissociates the antibody-therapeutic drug complexes within 2 min.

10.2.2. Incubate mixed samples and controls in reaction tubes or polypropylene microtiter plate for a maximum period of 20 min at room temperature (20-25^{o}C). Within this period of time (2-20 min) 40µL of 1:5 diluted-mixed sample and controls should be transferred into ELISA plate as described in 11.2 Assay Procedure.

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.
11.1.6. The total pipetting time needed for dispensing all samples into the wells should not exceed 5 minutes. If this is difficult to achieve the samples should be pre-dispensed in a separate neutral polypropylene microplate and then transferred into the reaction ELISA plate by a multi channel pipette.

11.2. ASSAY PROCEDURE

1.	Pipette **100 µl** of **Assay Buffer** into each of the wells to be used.
2.	Pipette **50 µl** of **Enzyme Conjugate** (HRP-Infliximab) into each well.
3.	Pipette **40 µL** of dissociation buffer-mixed **(as described in section 10.2) Negative Control, Positive Control and Samples** into the respective wells of the microtiter ELISA plate. Bubble formation during the pipetting of standards and samples must be avoided. <u>Wells</u> A1: Negative Control B1: Negative Control C1: Positive Control D1 and so on: Samples (Serum/Plasma)
4.	Cover the plate with adhesive seal. Shake plate carefully by tapping several times. **Incubate the plate on a bench top for 60 min** at **room temperature (RT, 20-25°C)**.
5.	Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate **5 X 350 µL** of **Diluted Wash Buffer** per well. Remove excess solution by tapping the inverted plate on a paper towel.
6	Pipette **100 µL** of Ready-to-Use **TMB** Substrate Solution into each well.
7.	**Incubate 15 min** at RT. Avoid exposure to direct sunlight**.**
8.	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.
9.	Measure optical density (OD) with a photometer at **450 nm** (Reference at OD620 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 below 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

For the run to be valid, the OD450 nm of the 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 each individual OD results by the Cut-off OD value. The results are expressed in arbitrary units (tAU/mL).

Cut-off value = 2 x the mean OD450nm of Negative Control = 10 tAU/mL

Samples which have an equal and higher OD than the cut-off value are considered to be positive.

Range	Interpretation
≥ Cut-off (10 tAU/mL)	POSITIVE
< Cut-off (10 tAU/mL)	NEGATIVE

An example for semi-quantitative calculation for a positve sample

OD of sample = 0.480

The mean OD of Negative Control = 0.080

Cut-off value (10 tAU/mL) = 2 x 0.080 = 0.160

Result for the sample = 0.480/0.160 × 10 tAU/mL = 30 tAU/mL

12. ASSAY CHARACTERISTICS

12.1. SPECIFICITY

88 different naive samples have been measured for estimating the cut-off value. In order to avoid a sample from being reported as false positive the cut-off value was determined by 2 times of the mean of Negative Control. All 88 screened naive samples showed ODs (ranged from 0.053 to 0.105) lower than the 1,5 times of the mean OD of Negative Control.

12.2. PRECISION

Intra-assay CV: <10%.
Inter-assay CV: <10%

13. AUTOMATION

Experiments have shown that the *ImmunoGuide* Total Antibody to Infliximab ELISA is suitable also for using by an automated ELISA processor.

14. REFERENCES

1. Malickova K, Duricova D, Bortlik M, Hind'os M, Machkova N, Hruba V, Lukas M, Zima T, Lukas M. Serum trough infliximab levels: A comparison of three different immunoassays for the monitoring of CT-P13 (infliximab) treatment in patients with inflammatory bowel disease. Biologicals.2015. pii: S1045-1056(15)00110-4. doi: 10.1016/j.biologicals.2015.09.005.

2. Minar P, Saeed SA, Afreen M, Kim MO, Denson LA. Practical Use of Infliximab Concentration Monitoring in Pediatric Crohn's Disease. J Pediatr Gastroenterol Nutr. 2015 Nov 5.doi: 10.1097/MPG.0000000000001029

3. Plasencia C, Jurado T, Villalba A, Peitedado D, Casla MT, Nuño L, Bonilla MG, Martínez-Feito A, Martín-Mola E, Pascual-Salcedo D, Balsa A. Effect of Infliximab Dose Increase in Rheumatoid Arthritis at Different Trough Concentrations: A Cohort Study in Clinical Practice Conditions. Front Med (Lausanne). 2015 Oct 8;2:71. doi: 10.3389/fmed.2015.00071.

4. Van Stappen T, Billiet T, Vande Casteele N, Compernolle G, Brouwers E, Vermeire S, Gils A. An Optimized Anti-infliximab Bridging Enzyme-linked Immunosorbent Assay for Harmonization of Anti-infliximab Antibody Titers in Patients with Inflammatory Bowel Diseases. Inflamm Bowel Dis. 2015;21(9):2172-7.

5. Hock BD, Stamp LK, Hayman MW, Keating PE, Helms ET, Barclay ML Development of an ELISA based competitive binding assay for the analysis of drug concentration and anti-drug antibody levels in patients receiving adalimumab or infliximab. Ther Drug Monit. 2015 Jul 24. [Epub ahead of print]

6. Ben-Horin S, Yavzori M, Benhar I, Fudim E, Picard O, Ungar B, Lee S, Kim S, Eliakim R, Chowers Y. Cross-immunogenicity: antibodies to infliximab in Remicade-treated patients with IBD similarly recognise the biosimilar Remsima. Gut. 2015 Apr 20. pii: gutjnl-2015-309290. doi: 10.1136/gutjnl-2015-309290.

7. Ungar B, Anafy A, Yanai H, Ron Y, Yavzori M, Picard O, Fudim E, Loebstein R, Kopylov U, Chowers Y, Dotan I, Eliakim R, Ben-Horin S. Significance of low level infliximab in the absence of anti-infliximab antibodies. World J Gastroenterol. 2015;21(6):1907-14.

8. Elberdín L, Outeda M, Salvador P, Paradela S, Fernández-Torres RM, Iglesias R, Fonseca E, Martín I. Infliximab drug and antibody levels in patients with dermatological conditions. Int J Clin Pharm. 2015;37(2):320-6.
9. Van Stappen T, Brouwers E, Tops S, Geukens N, Vermeire S, Declerck PJ, Gils A Generation of a Highly Specific Monoclonal Anti-Infliximab Antibody for Harmonization of TNF-Coated Infliximab Assays. Ther Drug Monit. 2015;37(4):479-85.
10. Hernández-Flórez D, Valor L, de la Torre I, Nieto JC, Martínez-Estupiñán L, González C, López-Longo FJ, Monteagudo I, Garrido J, Naredo E, Carreño L. Comparison of two ELISA versions for infliximab serum levels in patients diagnosed with ankylosing spondylitis. Rheumatol Int. 2015;35(6):1021-5.
11. Marinari B, Botti E, Bavetta M, Spallone G, Zangrilli A, Talamonti M, Richetta A, Chimenti S, Costanzo A. Detection of adalimumab and anti-adalimumab levels by ELISA: clinical considerations. Drug Dev Res. 2014;75 Suppl 1:S11-4.
12. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing infliximab treatment failure in Crohn's disease: post hoc analysis of a randomized controlled trial. Am J Gastroenterol. 2014;109(7):1055-64.
13. Marits P, Landucci L, Sundin U, Davidsdottir L, Nilsson J, Befrits R, Wikström AC, Eberhardson M Trough s-infliximab and antibodies towards infliximab in a cohort of 79 IBD patients with maintenance infliximab treatment. J Crohns Colitis. 2014;8(8):881-9.
14. Ungar B, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, Kopylov U, Eliakim R, Ben-Horin S; ABIRISK consortium. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. Gut. 2014;63(8):1258-64.
15. Corstjens PL, Fidder HH, Wiesmeijer KC, de Dood CJ, Rispens T, Wolbink GJ, Hommes DW, Tanke HJ A rapid assay for on-site monitoring of infliximab trough levels: a feasibility study. Anal Bioanal Chem. 2013;405(23):7367-75.
16. Steenholdt C, Ainsworth MA, Tovey M, Klausen TW, Thomsen OO, Brynskov J, Bendtzen K Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. Ther Drug Monit. 2013;35(4):530-8.
17. Imaeda H, Takahashi K, Fujimoto T, Bamba S, Tsujikawa T, Sasaki M, Fujiyama Y, Andoh A Clinical utility of newly developed immunoassays for serum concentrations of adalimumab and anti-adalimumab antibodies in patients with Crohn's disease. J Gastroenterol. 2014;49(1):100-9.
18. Wang SL, Ohrmund L, Hauenstein S, Salbato J, Reddy R, Monk P, Lockton S, Ling N, Singh S Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. J Immunol Methods. 2012;382(1-2):177-88.
19. Kopylov U, Mazor Y, Yavzori M, Fudim E, Katz L, Coscas D, Picard O, Chowers Y, Eliakim R, Ben-Horin S . Clinical utility of antihuman lambda chain-based enzyme-linked immunosorbent assay (ELISA) versus double antigen ELISA for the detection of anti-infliximab antibodies. Inflamm Bowel Dis. 2012;18(9):1628-33.
20. Imaeda H, Andoh A, Fujiyama Y . Development of a new immunoassay for the accurate determination of anti-infliximab antibodies in inflammatory bowel disease. J Gastroenterol. 2012;47(2):136-43.