



innovation for health & wellness

“trace & catch”

Instruction for Use

Antibody to Infliximab (Remicade®)

SHIKARI® Q-ATI

Enzyme immunoassay for the qualitative determination (screening) of antibodies to infliximab in serum and plasma

REF TR-ATIv5



12 x8



2-8 C

Revision # 5.1 December 2016



Matriks Biotek Laboratories
www.matriksbiotek.com

Contents	Page
Intend to use.....	3
Summary and Explanation.....	3
Test Principle	3
Warnings and Precautions	4
Storage and Stability.....	5
Specimen Collection and Storage.....	5
Materials Supplied.....	6
Materials Required but not Supplied	7
Procedure Notes.....	7
Preparation of Component	8
Test Procedure.....	9
Interpretation of Results.....	10
References.....	11

	SHIKARI Q-ATI
	Infliximab (Remicade®) antibodies qualitative analyse
Required Volume (µl)	10
Total Time (min)	140
Sample	Serum, plazma
Sample Number	96
Dedection Limit (ng/mL)	+/-
Spike Recovery (%)	-
Shelf Life (year)	1

Intended Use

The Matriks Biotek Antibody to infliximab (Remicade®) Enzyme-Linked-ImmunoSorbent-Assay (ELISA) Kit is intended for the qualitative determination of antibodies to infliximab (Remicade®) in serum and plasma. It is for professional use only.

Summary and Explanation

Infliximab (Remicade®) is a chimeric monoclonal antibody and used to treat autoimmune 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 anti-infliximab antibodies during treatment with infliximab (Remicade®) 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 Matriks Biotek ATI 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 to reduce anti-infliximab antibodies. With this Matriks Biotek ELISA test, antibodies to infliximab can be detected in patients receiving Remicade®.

Test Principle

The Matriks Biotek Antibody to infliximab (Remicade®) ELISA is a sandwich assay 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 (Remicade®) coated on the wall of the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled specific conjugate 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 color is directly proportional to the concentration of ATI in sample.

Warnings and Precautions

1. 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 Matriksbiotek 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.

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.

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
Stability:	7 d	6 mon	Avoid repeated freeze-thaw cycles

*. 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. Matriks Biotek Laboratories propose to obtain blood sample just before the infusion of infliximab (Remicade®) or at least 2 weeks after the infusion of infliximab (Remicade®).

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.
1 x 0.25 mL	RCTV CNTR	Reactive Control Ready-to-use. Contains infliximab -reactive antibody, human serum, stabilizers and <0.1% NaN ₃
1 x 0.5 mL	NEG CNTR	Negative Control Ready-to-use. Contains human serum, stabilizers and <0.1% NaN ₃
1 x 12 mL	ASSAY BUF	Assay Buffer Blue colored. Ready to use. Contains proteins, RF blockers and <0.1% NaN ₃
1 x 12 mL	POD CONJ	Peroxidase Conjugate Red colored. Ready to use. Contains peroxidase (POD) conjugate, RF blockers, 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	WASHBUF CONC	Wash Buffer, Concentrate (20x) Contains Buffer with Tween 20.
2 x 1	ADH FILM	Adhesive Film For covering of Microtiter Plate during incubation.

Materials Required but not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 μ L.
2. Bidistilled or deionised water
3. Calibrated measures.
4. Absorbent paper and timer.
5. Standard laboratory glass or plastic vials, cups, etc.
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450/650 nm (reference wavelength 620-690 nm is optional).

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.

Preparation of Component

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	3 w

*. Prepare Wash Buffer before starting assay procedure.

Test Procedure

1	Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used.
2	<p>QUALITATIVE ELISA TEST FORMAT</p> <p>Pipette 10 µL of ready-to use Cut-Off Serum, Reactive Control, and Samples into the respective wells of microtiter plate.</p> <p><u>Wells</u></p> <p>A1: Negative Control B1: Negative Control C1: Reactive Control D1 and on.: Sample (Serum/Plasma)</p>
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 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 foil.) at room temperature (18-25°C) in the dark.
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 with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution.

Interpretation Of Results

For the run to be valid, the OD_{450/650} nm of Positive Control should be ≥ 1.00 and the OD_{450/650} nm of each Negative Control should be < 0.2000 , if not, improper technique or reagent deterioration may be suspected and the run should be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD_{450/650} nm of the negative controls by 3.

I.e.;

If "Sample OD_{450/650} the mean OD_{450/650} of Negative Controls" is ≥ 3 , the sample is POSITIVE

If "Sample OD_{450/650} the mean OD_{450/650} of Negative Controls" is < 3 , the sample is NEGATIVE

REFERENCES

1. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Bijl H, Woody JN, Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis, *Lancet*, 1994; Oct 22;344(8930):1125-7.
2. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, et al., Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet*, Oct 22;344(8930):1105-10.
3. Keating GM, Perry CM, infliximab: an updated review of its use in Crohn's disease and rheumatoid arthritis, *BioDrugs*, 2002;16(2):111-48.
4. Lyseng-Williamson KA, Foster RH, infliximab: a pharmaco-economic review of its use in rheumatoid arthritis, *Pharmacoeconomics*, 2004;22(2):107-32.
5. Maini RN, Elliott MJ, Brennan FM, Williams RO, Chu CQ, Paleolog E, Charles PJ, Taylor PC, Feldmann M, Monoclonal anti-TNF alpha antibody as a probe of pathogenesis and therapy of rheumatoid disease, *Immunol Rev*, 1995 Apr;144:195-223.
6. Xu Z, Seitz K, Fasanmade A, Ford J, Williamson P, Xu W, Davis HM, Zhou H, Population pharmacokinetics of infliximab in patients with ankylosing spondylitis, *J Clin Pharmacol*, 2008 Jun;48(6):681-95. Epub 2008 Apr 9.
7. Han PD, Cohen RD, Managing immunogenic responses to infliximab: treatment implications for patients with Crohn's disease, *Drugs*, 2004;64(16):1767-77.
8. Caviglia R, Boskoski I, Cicala M, Long-term treatment with infliximab in inflammatory bowel disease: safety and tolerability issues, *Expert Opin Drug Saf*, 2008 Sep;7(5):617-32.
9. Reddy JG, Loftus EV Jr, Safety of infliximab and other biologic agents in the inflammatory bowel diseases, *Gastroenterol Clin North Am*, 2006 Dec;35(4):837-55.
10. Remicade approved for children with Crohn's disease, *FDA Comsum*, 2006 Jul-Aug;40(4):6.
11. Scheinfeld N, Off-label uses and side effects of infliximab, *J Drugs Dermatol*, 2004 MayJun;3(3):273-84.
12. Reimold AM, New indications for treatment of chronic inflammation by TNF-alpha blockade, *Am J Med Sci*, 2003 Feb;325(2):75-92.