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	SHIKARI S-AIR
	Infliximab (Remsima®) 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 biosimilar infliximab (Remsima®) Enzyme-Linked-ImmunoSorbent-Assay (ELISA) Kit is intended for the qualitative determination of antibodies to infliximab (Remsima®) in serum and plasma. It is for professional use only.

Summary and Explanation

Remsima™, the world first biosimilar mAb (approved in 2013 by EMA). The Agency's Committee for Medicinal Products for Human Use (CHMP) decided that, in accordance with EU requirements, Remsima has been shown to have a comparable quality, safety and efficacy profile to Remicade.

Remsima™ is a tumor necrosis factor α (TNF-α) antagonist used to treat rheumatoid arthritis, ankylosing spondylitis, ulcerative colitis, adult Crohn's disease, plaque psoriasis, and psoriatic arthritis.

Infliximab (Remsima®) is a chimeric monoclonal antibody and used to treat autoimmune disorders. Infliximab reduces the amount of active human tumour necrosis factor alpha (hTNFα) in the body 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 (Remsima®) 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 (Remsima®) 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 (Remsima®) 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 Remsima®.

Test Principle

The Matriks Biotek Antibody to infliximab (Remsima®) 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 (AIR) in patient serum/ plasma samples are captured by the drug infliximab (Remsima®) 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 AIR 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 (NaN3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN3 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 (Remsima®) 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 (Remsima®) or at least 2 weeks after the infusion of infliximab (Remsima®).

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.

Preparation of Component

Dilute/ disolve	Component	with	Diluent	Rela-tion	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	**QUALITATIVE ELISA TEST FORMAT** Pipette 10 µL of ready-to use Cut-Off Serum, Reactive Control, and Samples into the respective wells of microtiter plate. Wells A1: Negative Control B1: Negative Control C1: Reactive Control D1 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 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 <0.200 0, 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 OD450/650 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 NEGATİVE

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