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| | SHIKARI Q-ATI |
|---|---|
| | Infliximab (Remicade® ) antibodies quantitative analyse |
| Required Volume (µl) | 20 |
| Total Time (min) | 140 |
| Sample | Serum, plazma |
| Sample Number | 96 |
| Detection Limit (ng/mL) | 15 |
| Spike Recovery (%) | Between 85-115 |
| Shelf Life (year) | 1 |

## Intended Use

The Matriks Biotek 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.

## 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 (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 (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 Matriks Biotek 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 (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®).**

**Interpretation of true and false positives**

$$\frac{OD_{(450/650)} \text{ sample} - OD_{(450/650)} \text{ sample w/confirmation reagent}}{OD_{(450/650)} \text{ sample}} \times 100 = \text{inhibition \%}$$

For true positive sample, inhibition should be equal or greater than 25%

Example:

If the $OD_{(450/650)}$ of the tested sample is 0.800 and after incubation of the same sample with confirmation reagent and retested and $OD_{(450/650)}$ of the sample found to be 0.200, then;

$$\frac{0.800 - 0.200}{0.800} \times 100 = 75\%$$ the sample is "true positive" for anti-drug antibody

**QUALITATIVE INTERPRETATION**

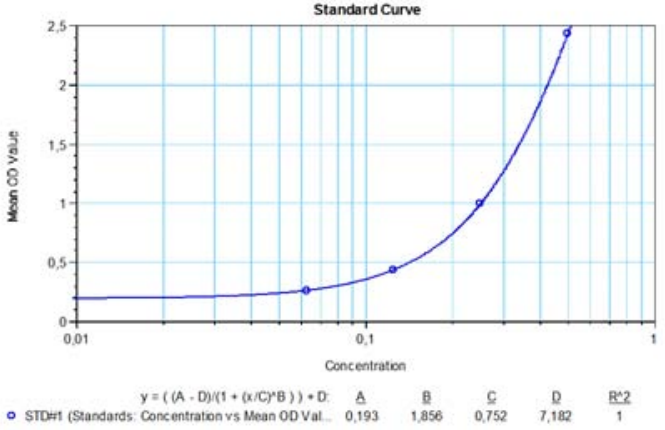
If "Sample $OD_{450/650}$ /Zero Standard (STD E/Negative Control) $OD_{450/650}$" is <3, the sample is NEGATIVE for ATI

If "Sample $OD_{450/650}$/Zero Standard (STD E/Negative Control) $OD_{450/650}$" is ≥3, the sample is POSITIVE for ATI and if required samples may be extrapolated for quantitative analysis and confirmation.

For the run to be valid, the $OD_{450/650}$ nm of Positive Control (Standard A) should be ≥ 1.500 and the $OD_{450/650}$ nm of each Negative Control should be <0.200, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.

**Typical Calibration Curve**

(Example. Do not use for calculation!)




| Standard | Concentration (ng/mL) | Mean $OD_{450/650}$ |
|---|---|---|
| A | 500 | 2,424 |
| B | 250 | 0,994 |
| C | 125 | 0,434 |
| D | 62 | 0,261 |
| E | 0 | 0,035 |

## REFERENCES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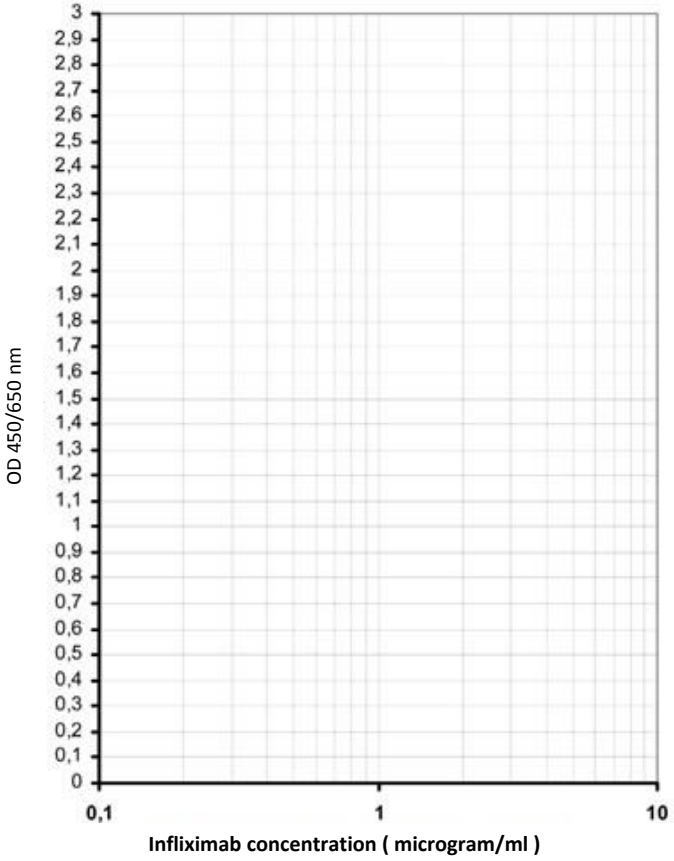
OD 450/650 nm
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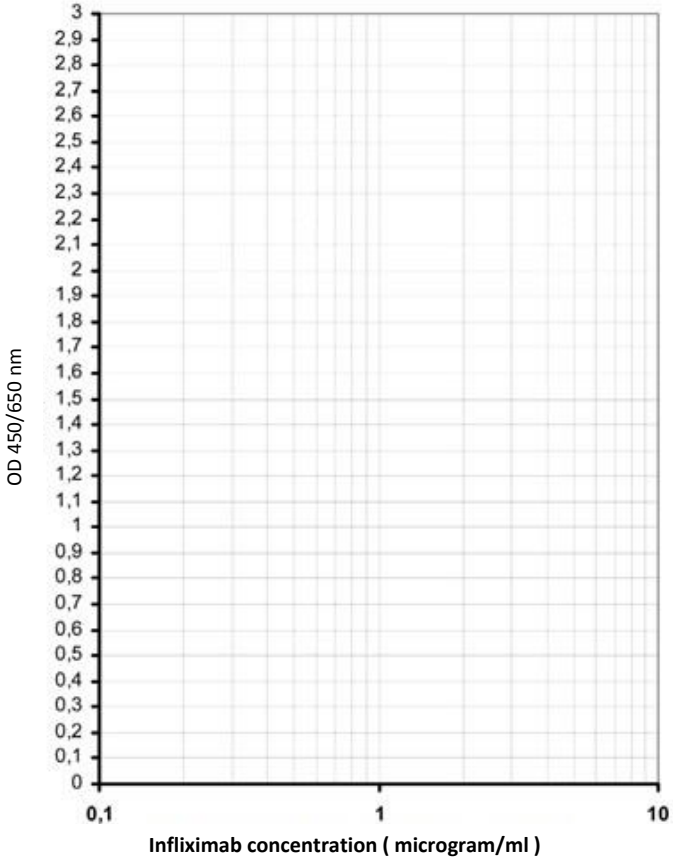
Infliximab concentration ( microgram/ml )


OD 450/650 nm
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Infliximab concentration ( microgram/ml )