MATRIKS BIOTEK

"trace & catch"

**Instructions for Use**

# SHIKARI® (S-ATAB)

## Qualitative Antibodies to Abatacept ELISA

**Enzyme immunoassay for determination of qualitative antibodies to Abatacept in serum and plasma samples**

| | |
|---|---|
| REF | **ABA-QLS-ORE** |
| Σ | 96 tests |
| | Shipment 10-30°C, Store 2-8°C |
| | MATRIKS BIOTECHNOLOGY CO., LTD.<br>Bahcelievler Mah. 323/1 Cad. Gazi Universitesi Teknokent<br>Binası C Blok No:10/50C/47 06830<br>Golbasi Ankara / TURKEY<br>Tel +90 312 485 42 94<br>info@matriksbiotek.com |
| CE IVD | |

## 1. Intended Use

SHIKARI® Qualitative Antibodies to Abatacept ELISA has been especially developed for the qualitative analysis of antibodies to Abatacept in serum and plasma samples. SHIKARI® Qualitative Antibodies to Abatacept ELISA is optimized with Orencia®.

## 2. General Information

Abatacept is licensed for the treatment of rheumatoid arthritis (RA) in combination with methotrexate. The indication includes moderate to severe RA unresponsive to other disease- modifying antirheumatic drugs including at least one tumour necrosis factor (TNF)- a blocker, or where patients have been intolerant of such drugs.
Abatacept (CTLA4Ig) is a fusion protein of the extracellular domain of the human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) linked to a modified Fc of human immunoglobulin 1 (IgG1). Abatacept inhibits the activation of Tlymphocytes that play an important role in the early stages of pathogenesis of RA. Activation of a T cell requires two signals from the antigen-presenting cell (APC).

The first signal is antigen specific and arises when antigenic peptides are presented to the T cell through the Major Histocompatibility Complex. A second signal, so-called co-stimulation, develops from the interaction between CD80 or CD86 antigen on the APC and CD28 antigen on the T cell. Abatacept binds with the extracellular domain of CTLA-4 to CD80 or CD86 antigen on the APC with a higher affinity than CD28, preventing the essential second signal for T-cell activation. T-cell activation and the production of inflammatory mediators and cytokines (TNF-a, interferon-gamma and interleukin-2) are consequently reduced. Trials in patients with RA have shown that abatacept slows progression of joint damage and improves function.
An increased incidence of all kinds of infections caused by abatacept is explained by its mechanism of suppressing the immune response. Headache, hypertension, dizziness, gastrointestinal disorders and rash are other common adverse effects. A small percentage of treated patients develop anti- abatacept antibodies.

Therapeutic drug monitoring (TDM) is the clinical practice of measuring specific drugs at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimizing individual dosage regimens. The indications for drug monitoring include efficacy, compliance, drug-drug interactions, toxicity avoidance, and therapy cessation monitoring. Additionally, TDM can help to identify problems with medication compliance among noncompliant patient cases.

Biologic medicinal products (biologics) have transformed treatment landscapes worldwide for patients with haematological or solid malignancies with the 21st century. Today, as data exclusivity periods of first wave biologics approach expiration/have expired, several biosimilar products (i.e.,biologics that are considered to be similar in terms of quality, safety and efficacy to an approved 'reference' biologic) are being developed or have already been approved for human use.

Like all biologics, biosimilars are structurally complex proteins that are typically manufactured using genetically engineered animal, bacterial or plant cell culture systems. As a consequence of this molecular complexity and the proprietary nature of the manufacturing process, which will inevitably result in the use of different host cell lines and expression systems as well as related differences in manufacturing conditions, it is not possible to manufacture exact copies of a reference biologic.

When administered to patients, all therapeutic proteins have the potential to induce an unwanted immune response (i.e.,to stimulate the formation of antidrug antibodies [ADAs]). The impact of immune responses can range from no apparent effect to changes in pharmacokinetics, loss of effect and serious adverse events. Furthermore, the immunogenicity profile of a biologic can be significantly altered by even small differences in its manufacturing process that are accompanied by a change in product attributes, as well as differences in dosing schedules, administration routes or patient populations.

SHIKARI® ELISA kits can be used for drug level and anti-drug antibodies measurements. SHIKARI® Abatacept ELISA products:

| Brand | Description | | Product Code |
|---|---|---|---|
| SHiKARi® (Q-ABA) | Abatacept | Free Drug | **ABA-FD-ORE** |
| SHiKARi® (S-ATAB) | Abatacept | Antibody screening - Qualitative | **ABA-QLS-ORE** |
| SHiKARi® (S-ATAB) | Abatacept | Antibody screening - Quantitative | **ABA-QNS-ORE** |

Check the web page for the whole product list **www.matriksbiotek.com**

## 3. Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Controls and samples (serum or plasma) are incubated in the microtiter plate coated with the drug abatacept. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to abatacept antibodies captured by the drug abatacept on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed is proportional to the amount of abatacept antibodies in the sample or controls. The results can be evaluated with using cut-off value.

## 4. Warnings and Precautions

- For professional use only.

- 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.

- Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

- 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.

- Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

- All reagents of this kit containing human serum or plasma (standards etc.) have been tested and were found negative for HIV I/II, HBsAg and Anti-HCV. 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.

- Reagents of this kit containing hazardous material may cause eye and skin irritations. See "Materials supplied", SDS and labels for details.

- Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations

## 5. Storage and Stability

The kit is shipped at ambient temperature (10-30°C) and should be stored at 2-8°C for long term storage. Keep away from heat or direct sunlight. The strips of microtiter plate 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 and Storage)

Serum, Plasma (EDTA, Heparin)

The usual precautions for venipuncture should be observed. Do not use grossly haemolytic, icteric or lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Avoid repeated freeze- thaw cycles for serum/plasma samples.

Samples should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test.

Drug infusions may camouflages/mask the presence of antibody to drugs in serum/plasma samples. Therefore, blood sampling time is critical for detection of antibodies. It is recommended to take the blood sample just before the scheduled dose (trough specimen).

| Storage | 2-8°C | -20°C |
|---|---|---|
| Stability (serum/plasma) | 2 days | 6 months |

## 7. Materials Supplied

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|---|---|---|
| **Microtiter Plate** | 1 x 12 x 8 | **Microtiter plate**<br>Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with Abatacept. |
| **Controls** | 1 mL (each) | **Control Negative & Positive**<br>Ready to use. Contains human serum and stabilizer, <0,1% $NaN_3$ |
| **Assay Buffer** | 1 x 50 mL | **Assay buffer**<br>Ready to use. Blue coloured.<br>Contains proteins, <0,1 % $NaN_3$ |

| | | |
|---|---|---|
| **Conjugate** | 1 x 12 mL | **Horse radish peroxidase conjugated probe**<br>Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives. |
| **Substrate** | 1 x 12 mL | **TMB substrate solution**<br>Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB) |
| **Stop Buffer** | 1 x 12 mL | **TMB stop solution**<br>Ready to use. 1N HCl |
| **Wash Buffer** | 1 x 50 mL | **Wash buffer (20x)**<br>Prepared concentrated (20x) and should be diluted with the dilution rate given in the "Pre- test setup instructions" before the test. Contains buffer with tween 20. |
| **Foil** | 2 x 1 | **Adhesive Foil**<br>For covering microtiter plate during incubation |

## 8. Materials Required but Not Supplied

- Micropipettes and tips

- Calibrated measures

- Tubes for sample dilution

- Wash bottle, automated or semi-automated microtiter plate washing system

- Microtiter plate reader capable of measuring optical density with a photometer at OD 450nm with reference wavelength 650 nm (450/650 nm)

- Distilled or deionised water, paper towels, pipette tips and timer

## 9. Procedure Notes

- 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 must be performed strictly according to the instructions. Use calibrated pipettes and devices only.

- 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.

- 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.

- Use a pipetting scheme to verify an appropriate plate layout.

- Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an eight-channel micropipette for pipetting of solution in all wells.

- 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.

- 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**

- Preparation of components

| **Component** | Wash buffer<br>(Must be prepared before starting assay procedure) |
|---|---|
| **Dilute** | 10 mL (e.g.) |
| **With** | Up to 200 mL |
| **Diluent** | Distilled water |
| **Dilution Ratio** | 1/20 |
| **Remarks** | Warm up 37°C to dissolve crystals. Mix vigorously |
| **Storage** | 2-8°C |
| **Stability** | 2 weeks |

- Dilution of samples

| | |
|---|---|
| **Sample** | Serum/Plasma |
| **Diluent** | Assay buffer |
| **Dilution Ratio** | 1/10 |
| **Remarks** | 1/10 dilution<br>20 µL sample + 180 µL assay buffer |

Patient samples with a concentration of drug above the measuring range are to be rated as > "Highest Standard (Standard A)". The result must not be extrapolated. The patient sample in question should be further diluted with assay buffer and retested.

**11. Test Procedure**

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|---|---|
| | Total assay time: 140 minutes |
| **1** | Pipette 100 µL of each "Negative control", "Positive control" and diluted samples into the respective wells of microtiter plate.<br><br>**Wells**<br>A1: Negative control*<br>B1: Negative control*<br>C1: Positive control<br>D1 and on: Samples<br><br>*It is advised to run more than one "Negative control" samples. Negative control studies can be duplicated or triplicated in order to take the mean value. |
| **2** | Cover the plate with adhesive foil.<br>Briefly mix contents by gently shaking the plate.<br>Incubate 60 minutes at room temperature (18-25°C). |
| **3** | Remove adhesive foil .<br>Discard incubation solution.<br>Wash plate three times each with 300 µL "Wash Buffer".<br>Remove excess solution by tapping the inverted plate on a paper towel. |
| **4** | Pipette 100 µL "Conjugate" into each well. |

| | |
|---|---|
| **5** | Cover the plate with adhesive foil.<br>Incubate 60 minutes at room temperature (18-25°C). |
| **6** | Remove adhesive foil .<br>Discard incubation solution.<br>Wash plate three times each with 300 µL "Wash Buffer".<br>Remove excess solution by tapping the inverted plate on a paper towel. |
| **7** | Pipette 100 µL "Substrate" into each well. |
| **8** | Incubate 20 minutes without adhesive foil at room temperature (18-25°C) in the dark. |
| **9** | Stop the substrate reaction by adding 100 µL "Stop Solution" into each well.<br>Briefly mix contents by gently shaking the plate .<br>Colour changes from blue to yellow. |
| **10** | Measure optical density with a photometer at OD 450 nm with reference wavelength 650 nm (450/650 nm) within 30 minutes after pipetting the "Stop Solution". |

## 12. 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. For the run to be valid, the OD 450/650 nm of positive control should be >1,000 and the OD 450/650 nm of each negative control should be <0,200. In case of any deviation the following technical issues (but not limited to) should be reviewed: Expiration dates of reagents, storage conditions, pipettes, devices, incubation conditions, washing methods, etc.

## 13. Calculation and Interpretation of Results

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.

e.g.

If "Sample OD 450/650 / the mean negative control OD 450/650 ≥ 3" then the sample is POSITIVE

If "Sample OD 450/650/ the mean negative control OD 450/650 <3" then the sample is NEGATIVE

Note: The cut-off information provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories.

## 14. Analytical Performance

- Specificity: There is no cross reaction with native serum immunoglobulin

- Precision: Intra-assay and inter-assay CVs <30%

- Cut-off: Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories.

The "Quality control certificate" contains lot specific analytical performance data and is supplied separately with each kit. If some further analytical performance data is needed, please refer to the local distributor.

## 15. Automation

SHIKARI® Qualitative Antibodies to Abatacept ELISA is also suitable to run on automated ELISA processors.

## 16. Symbols and Cautions

| | | | |
|---|---|---|---|
|  | Manufacturer |  | Temperature limitation |
|  | Production date |  | See instruction for use |
|  | Expiry date |  | Caution |
|  | Lot number |  | In vitro diagnostic medical device |
|  | Catalog number |  | Control |
|  | Do not use if package is damaged |  | Negative control |
|  | Keep away from sunlight |  | Positive control |
|  | Keep dry |  | Number of tests |

*According to ISO 15223*

**Cautions:** The performance of the kit can be achieved by fully complying with the instructions. Modifications on the test procedure can affect the results and these kinds of changes will not be charged as regular complaints. This product is for professional use only and must be used for "Intended use" that is given in the instructions for use. The results themselves should not be the only reason for any therapeutically consequences. They must be correlated to other clinical observations. Cut-off, reference ranges, etc. must be calculated/set according to scientific standards by the users/laboratories. Information in the instructions about cut- off, etc. performance characteristics, can only be considered as a recommendation and does not give any responsibility to the manufacturer.

**Limitations of liability:** The manufacturer's liability is limited to the purchase price of the product in all circumstances. The manufacturer cannot be held responsible for damage to the patient, lost profit, lost sales, damage to property or any other incidental or consequential loss.

**Technical support and complaints:** Technical support can be given upon request. If there is a problem with the product, complaints must be sent written to info@matriksbiotek.com with the technical data (if available) like standard curve, control results, etc. After the necessary examination, written reply will be given.

## 17. References

- Moreland L, Bate G, Kirkpatrick P. Fresh from the pipeline: Abatacept. Nat Rev Drug Discov 2006; 5: 185–6

- Orencia FDA Label

## 18. Revision summary

| Revision no | Release date | Explanation |
|---|---|---|
| 01 | 06.03.2020 | New Documentation |
| 02 | 14.10.2021 | Change Test Procedure |
| 03 | 22.12.2022 | Sections 7, 10, 11, 12 and 14 have been revised. Document code has been changed. |
| 04 | 17.07.2023 | Company address has been revised. Company logo has been changed. |

*Notes:*


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