MATRIKS BIOTEK
innovation for health & wellness

"trace & catch"

Instructions for Use

Antibody to Adalimumab (Humira®) ELISA

SHIKARI®
S-ATA

Enzyme immunoassay for the quantitative determination (screening) of antibodies to adalimumab (Humira®) in serum and plasma

REF **TR-AADAv2** Σ 12 x 8 2-8°C

Revision # 2.1 December 2016

CE
IVD

Matriks Biotek® Laboratories
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	SHIKARI S-ATA
	Adalimumab (Humira®) antibodies qualitative analyses
Required Volume (µl)	10
Total Time (min)	140
Sample	Serum, plasma
Sample Number	96
Detection Limit (ng/mL)	+/-
Spike Recovery (%)	-
Shelf Life (year)	1

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

*. Adalimumab (Humira®) infusion camouflages/masks the presence of antibody to adalimumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of adalimumab. Matriks Biotek® Laboratories propose to obtain blood sample just before the infusion of adalimumab (Humira®) or at least 2 weeks after the infusion of adalimumab (Humira®) .

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.

Pre-Test Setup Instructions

1. Preparation of Components

Dilute/ disolve	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	1 w

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

2. Dilution of Standards and Samples (serum/plasma)*

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Serum/Plasma	1/10	Assay Buffer	1:10 – 1:100	For dilution 1:10 10µl Sample + 90µl Assay Buffer For dilution 1:100 5µl Sample + 495µl Assay Buffer

*. Patient samples with a concentration of ATI above the measuring range are to be rated as "> highest standard". The result must not be extrapolated. The patient sample in question should be diluted with Assay Buffer and then retested.

PREPRATION OF CONFIRMATION TEST MIXTURE

Sample	To be diluted	With	Relation	Remarks
Serum/Plasma	Initially no	Confirmation Reagent*	1:10	For dilution 1:10 10µL Sample + 90µL Confirmation Reagent

Test Procedure

1	**QUANTITATIVE ELISA TEST FORMAT** Pipette 100 µL of ready-to use **Standards, Samples and Confirmation test mixture** into the respective wells of microtiter plate. Wells A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E (Negative Control) F1 and on: Sample (Serum/Plasma)
2	Cover the plate with adhesive film. Briefly mix contents by gently shaking the plate. **Incubate 60 min** at room temperature (18-25°C).
3	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.
4	Pipette 100 µL of ready-to use **Peroxidase Conjugate** into each well.
5	Cover the plate with adhesive film. **Incubate 60 min** at room temperature (18- 25°C).
6	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.
7	Pipette **100 µL** of **TMB Substrate Solution** into each well.
8	**Incubate 20 min** (without adhesive foil.) at room temperature (18-25°C) in the dark.
9	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
10	Measure optical density with a photometer at **450/650 nm** within **30 min** after pipetting of the Stop Solution.

Confirmation Test

	CONFIRMATION TEST FOR POSITIVE SAMPLES
	Incubate positive patient samples and optimized confirmation reagent for **60 minutes in a microtube**. After the incubation proceed the test procedure from step one given above.

QUALITY CONTROL

The test results are only valid only 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 must be found within the acceptable ranges as stated above. 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 proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation & Interpretation of Results

1. A standard curve should be calculated using the standard concentration (X-axis) versus the OD450 (we suggest that OD readings done at double wavelength at OD450/650 and OD value read at 650nm subtracted from 450/650 nm OD VALUE) VALUES (Y-axis). In case of manual plot, we suggest semilog graph paper. If computer data regation is going to be used, we recommend primarily "4 Parameter Logistic (4PL)" or secondly the "point-to-point calculation".

2. The concentration of the samples can be read from this standard curve as follows. Using the absorbance value for each sample, determine the corresponding concentration of the drug from standard curve. This value always has to be multiplied by the dilution factor. If any diluted sample is reading greater than highest standard, it should be further diluted appropriately with Assay Buffer and retested. Also this second dilution has to be used for calculation the final results.

3. Any sample diluted at 1:100 and still reading greater than the highest standard should be further diluted appropriately with Assay Buffer and retested. **Because the standards are also diluted at 1:100 just as the samples, the concentration determined from the standard-curve directly represents the serum bevacizumab level.**

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