MATRIKS
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innovation for health & wellness

Instruction for Use

Antibody to CT-P13 (Infliximab biosimilar)

SHIKARI®
S-AIR

Enzyme immunoassay for the quantitative determination of antibodies to infliximab biosimilar CT-P13 (Remsima ™, Inflectra ™) in serum and plasma

REF **TR-AREMSv2** $\sum$ 12 x8 $2\text{-}8^0\text{C}$

Revision # 2.1 December 2016

Matriks Biotek® Laboratories
www.matriksbiotek.com

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IVD

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	CT-P13 antibodies quantitavie analyse
Required Volume (µl)	10
Total Time (min)	140
Sample	Serum, plasma
Sample Number	96
Dedection Limit (ng/mL)	30ng/ml (+/-)
Spike Recovery (%)	%95
Shelf Life (year)	1

Intended Use

The Matriks Biotek Antibody to CT-P13 Enzyme-Linked-ImmunoSorbent-Assay (ELISA) Kit is intended for the quantitative determination of antibodies to CT-P13 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 CT-P13 (Remsima®) ELISA is a sandwich assay for the determination of antibodies against CT-P13 in serum and plasma samples. During the first incubation period, antibodies to CT-P13 in patient serum/ plasma samples are captured by the drug CT-P13 (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

*. CT-P13 (Remsima®) infusion camouflages/masks the presence of antibody to CT-P13 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 CT-P13 (Remsima®) or at least 2 weeks after the infusion of CT-P13 (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 CT-P13.
5 x 1 mL	STND A-E	**ATI Standards A-E** 500; 250; 125; 62; 0 ng/mL Ready to use. Used for construction of the standard curve. Contains Antibody to Infliximab (ATI), human serum and <0,1% NaN_3
1 x 50 mL	ASSAY BUF	**Assay Buffer** Blue colored. Ready to use. Contains proteins, RF blockers and <0.1% NaN_3
1 x 12 mL	CONFIRMATION REAGENT	**Confirmation Reagent** Blue colored. Ready to use. Contains optimized concentration of the infiliximab, proteins, stabilizer, 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
2 x 1	ADH FILM	**Adhesive Film** For covering of Microtiter Plate during incubation.
2 x 1	SLGP	**Semi-Log Graph Paper** For constructing standard curve and calculation of results.

Materials Required But Not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
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.

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/ 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.

Sample	To be diluted	With	Relation	Remarks
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 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.**

QUALITATIVE INTERPRETATION

If "Samle $OD_{450/650}$/Zero Standard (STD E/Negative Control) $OD_{450/650}$" is <3, the sample is NEGATIVE for Anti CT-P13

If "Samle $OD_{450/650}$/Zero Standard (STD E/Negative Control) $OD_{450/650}$" is ≥3, the sample is POSITIVE for Anti CT-P13 and should be extrapolated for quantitative analysis.

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.
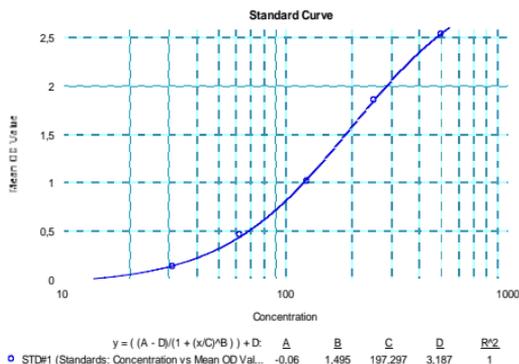
QUANTITATIVE INTERPRETATION

1. Using the standards (500, 250, 125, 62 ng/mL), construct a standard curve byplotting the OD450/650 nm for each of 4 standards on the vertical (Y-axis) axis versus the corresponding Anti CT-P13 concentration on the horizontal (X-axis) axis, thus creating a standard curve by 4 points obtained.

2. The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of Anti CT-P13 from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the Anti CT-P13 concentration for the unknown sample.

3. Any sample reading greater than the highest standard should be diluted appropriately with Assay Buffer and retested. Therefore, if the samples have been diluted, the concentration determined from the standard-curve must be multiplied by the dilution factor.

4. Automated method: Computer programs can also generally give a good fit.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Concentration (ng/mL)	Mean OD$_{450/650}$
A	500	2,536
B	250	1,859
C	125	1,011
D	62	0,454
E	0	0,033

Standard Curve

$y = ((A - D)/(1 + (x/C)^B)) + D$:

	A	B	C	D	R^2
STD#1 (Standards: Concentration vs Mean OD Val...	-0,06	1,495	197,297	3,187	1

Assay Characteristics

1. **Specificity**: CT-P13 (Remsima®) infusion camouflages/masks the presence of antibody to CT-P13(Anti CT-P13) in serum/plasma samples. Therefore, blood sampling time is critical for detection of Anti CT-P13. It is convenient to obtain blood sample just before the infusion of CT-P13 (Remsima®) or at least 2 weeks after the infusion of CT-P13 (Remsima®).

2. **Sensitivity:** The lowest detectable level that can be distinguished from the zero standard is <30 ng/mL.

3. **Precision of Kit:**
   Intra-assay CV: <20% for the Anti CT-P13 range of 62-500 ng/mL.
   Inter-assay CV: <20% for the Anti CT-P13 range of 62-500 ng/mL.

4. **Recovery:** Recovery rate was found to be higher than 97% when spiked using normal human serum samples with known concentrations.

Automation

Experiments have shown that the Matriks Biotek S-AIR ELISA is also suitable to run on

an automated ELISA processor.

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Infliximab biosimilar CT-P13 concentration (microgram/ml)

y-axis label: OD 450/650 nm

Y-axis label: OD 450/650 nm

X-axis label: **Infliximab biosimilar CT-P13 concentration (microgram/ml)**