Technical Manual

Infliximab ELISA Kit (Remsima®) Semi-Quantitative

- Catalogue Code: HUMB00008
- Research Use Only

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1. Intended Use

The Assay Genie Semi-Quantitative Free/Total Antibodies to Infliximab Biosimilar ELISA has been especially developed for the semi-quantitative analysis of free and total antibodies to infliximab biosimilar in serum and plasma samples. Assay Genies' Semi-Quantitative Free/Total Antibodies to Infliximab Biosimilar ELISA is optimized with Remsima®.

2. General Information

Infliximab is a tumour necrosis factor (TNFα) blocker and a chimeric monoclonal IgG1 antibody composed of human constant (75%) and murine variable (25%) regions. Infliximab is produced by a recombinant cell line cultured by continuous perfusion. TNFα is a key proinflammatory cytokine involved in chronic inflammatory diseases. Its hyperactivity and enhanced signalling pathways can be observed in inflammatory diseases where it activates further pro-inflammatory cascades. By binding to both the soluble subunit and the membrane-bound precursor of TNFα, infliximab disrupts the interaction of TNFα with its receptors and may also cause lysis of cells that produce TNFα.

Infliximab is an IgG1κ monoclonal antibody that binds to soluble and transmembrane forms of TNFα with high affinity to disrupt the pro-inflammatory cascade signalling. Binding of the antibody to TNFα prevents TNFα from interacting with its receptors. Infliximab does not neutralize TNFα (lymphotoxin-α), a related cytokine that utilizes the same receptors as TNFα. Blocked actions of TNFα further leads to downregulation of local and systemic proinflammatory cytokines (i.e. IL-1, IL-6), reduction of lymphocyte and leukocyte migration to sites of inflammation, induction of apoptosis of TNF-producing cells (i.e. activated monocytes and T lymphocytes), increased levels of nuclear factor-κB inhibitor, and reduction of reduction of endothelial adhesion molecules and acute phase proteins. Its inhibitory actions on TNFα was demonstrated in human fibroblasts, endothelial cells, neutrophils, B and T lymphocytes and epithelial cells. Infliximab also attenuates the production of tissue degrading enzymes synthesized by synoviocytes and/or chondrocytes.

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.

Assay Genies' ELISA kits can be used for drug level and anti-drug antibodies measurements. Infliximab ELISA products:

Kit Name	Description	Product SKU
Infliximab (Remicade®) ELISA Kit	Free drug	HUMB00001
Anti-Infliximab (Remicade®) ELISA Kit	Antibody screening - Qualitative	HUMB00002
Anti-Infliximab (Remicade®) ELISA Kit	Antibody screening - Quantitative	HUMB00003
Anti-Infliximab (Remicade®) ELISA Kit	Antibody screening – Free/Total semi-quantitative	HUMB00004
Infliximab (Remsima®) ELISA Kit	Free drug	HUMB00005
Anti-Infliximab (Remsima®) ADA ELISA Kit	Antibody screening - Qualitative	HUMB00006
Anti-Infliximab (Remsima®) ADA ELISA Kit	Antibody screening - Quantitative	HUMB00007
Anti-Infliximab (Remsima®) ADA ELISA Kit	Antibody screening – Free/Total semi-quantitative	HUMB00008

3. Test Principle

Assay Genies' Semi-Quantitative Free/Total Antibodies to Infliximab Biosimilar ELISA is a sandwich assay for the determination of total and free antibodies against infliximab in serum and plasma samples. During the first incubation period, the separation of infliximab specific antibody-infliximab immune complex is provided by adding dissociation buffer. After transferring dissociation mix to the plate, infliximab antibodies are separated from infliximab in patient serum/plasma samples and they are captured by the drug infliximab coated on the wall of the microtiter wells and horse radish peroxidase (HRP) conjugated probe. After washing away the unbound components from samples, 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 colour is directly proportional to the concentration of infliximab antibodies in sample.

Assay Genies' Semi-Quantitative Free/Total Antibodies to Infliximab Biosimilar ELISA kit can be also used as a semi-quantitative test for free anti-drug antibodies determination without dissociation and neutralization steps. Peroxidase labelled specific conjugate and diluted serum/plasma samples are transferred simultaneously to the infliximab-coated plate and

antibodies to infliximab in patient serum/plasma samples are captured by the drug infliximab coated on the wall of the microtiter wells and HRP conjugated probe. After washing away the unbound components from samples, the bound enzymatic activity is detected by addition of TMB chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The intensity of the reaction colour is directly proportional to the concentration of infliximab antibodies in sample.

4. Warnings and Precautions

− For Research Use Only.

− In case of severe damage of the kit package please contact Assay Genie 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”, MSDS 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

Microtiter Plate	1 x 12 x 8	**Microtiter plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant.
Controls	1.0 mL (negative) 0.5mL (positive)	**Control negative and positive** Ready to use. Contains human serum and stabilizer, <0.1% NaN_3
Assay Buffer	1 x 50 mL	**Assay buffer** Ready to use. Blue coloured. Contains proteins, <0.1% NaN_3
Immune Complex Control	1 x 1.5mL	**Immune complex control** Ready to use. Contains anti-infliximab/infliximab immune complex, human serum and stabiliser. <0,1% NaN_3
Dissociation Buffer	1 x 25mL	**Dissociation buffer** Ready to use. Contains diluted acid
Neutralisation Buffer	1 x 5 mL	**Neutralisation buffer** Ready to use
Conjugate	1 x 12 mL	**Horse radish peroxidase conjugated probe** Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.
Confirmation Reagent	1 x 12 mL	**Confirmation reagent** Ready to use. Contains proteins, infliximab and stabilizer, 0,1% NaN_3
Substrate	1 x 12 mL	**TMB substrate solution** Ready to use. Contains 3,3′,5,5′- Tetramethylbenzidine (TMB)
Stop Buffer	1 x 12 mL	**TMB stop solution** Ready to use. 1N HCl
Wash Buffer	1 x 50 mL	**Wash buffer (20x)** 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** 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 solutions 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 (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 to 37°C to dissolve crystals. Mix Vigorously.
Storage	2-8°C
Stability	2 weeks

-Dilution of samples and controls for free antibodies

Sample	Serum/Plasma	Controls
Diluent	Assay buffer	Assay Buffer
Dilution Ratio	1/5	1/5
Remarks	1/5 dilution 40 µL sample + 160 µL assay buffer	1/5 dilution 40 µL control + 160 µL assay buffer

-Preparation of immune complex control for total antibodies

Sample	Immune complex control
Diluent	Assay buffer
Dilution Ratio	1/5
Remarks	1/5 dilution 40 µL control + 160 µL assay buffer

11. Test Procedure

Total Antibodies to Infliximab	Free Antibodies to Infliximab
Total assay time: 95 minutes	Total assay time: 80 minutes
Pipette 40 µL of each “Negative control”, “Positive control” and “Immune complex control” and samples into the respective tubes	
Add 160 µL “Dissociation buffer” to tubes and incubate the plate 15 minutes at room temperature (18- 25°C)	
Pipette 65 µL “Peroxidase conjugate” and 35 µL “Neutralisation buffer” into each of the wells to microtiter plate and transfer 100 µL dissociation mix into each of the respective wells of microtiter plate Wells A1: Negative control* B1: Negative control* C1: Positive control D1: Immune complex control (after acid dissociation) E1: Immune complex control (before acid dissociation, diluted with assay buffer) F1 and on: Samples *It is advised to run more than one “Negative control” samples and. Negative control studies can be duplicated or triplicated in order to take the mean value	Pipette 65 µL “Peroxidase conjugate into each of the wells to microtiter plate and transfer 135 µL of diluted “Negative control”, “Positive control” and samples into each of the respective wells of microtiter plate Wells A1: Negative control* B1: Negative control* C1: Positive control D1 and on: Samples *It is advised to run more than one “Negative control” samples and. Negative control studies can be duplicated or triplicated in order to take the mean value

Cover the plate with adhesive foil
Briefly mix contents by gently shaking the plate
Incubate 60 minutes at room temperature (18-25°C)

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Remove adhesive foil
Discard incubation solution
Wash plate three times each with 300 µL “Wash Buffer”
Remove excess solution by tapping the inverted plate on a paper towel

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Pipette 100 µL “Substrate” into each well

↓

Incubate 20 minutes without adhesive foil at room temperature (18-25°C) in the dark

↓

Stop the substrate reaction by adding 100 µL “Stop Solution” into each well
Briefly mix contents by gently shaking the plate
Colour changes from blue to yellow

↓

Measure optical density with a photometer at OD 450nm 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 a valid study, the OD 450/650 of the highest standard should be >1.500 and the OD 450/650 of the lowest standard should be <0.150. 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. Calculations and Interpretation of Results

− The results are expressed in arbitrary units (AU/mL, antibody unit/mL)

− The results are evaluated by a cut-off calculation is as follows

Cut-off value: 2 x Negative control OD 450/650 nm = 10 antibody units (AU/mL)

Range	Interpretation
≥10 AU/mL	Positive
<10 AU/mL	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.

e.g.

Patients sample OD 450/650 nm: 0.600

The mean OD 450/650 nm of negative controls: 0.075

Cut-off value = 2 x 0.075 = 0.150 = 10 AU/mL

Result of the patient sample: 0.600 / 0.150 = 4 x 10 AU/mL = 40 AU/mL (Positive)

− Immune complex control interpretation

Acid dissociation (AD) control is done with immune complex control (ICC) results.

$$\frac{\text{After AD ICC OD 450/650 nm - Before AD ICC OD 450/650 nm}}{\text{Before AD ICC OD 450/650 nm}} \times 100 > 20\%$$

e.g.

Before AD ICC OD 450/650 nm: 0.286

After AD ICC OD 450/650 nm: 0.050

(0.286-0.050) / 0.050 x 100 = 472%

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

Assay Genies’ Semi-Quantitative Free/Total Antibodies to Infliximab Biosimilar 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
REF	Catalog number	CONTROL	Control
	Do not use if package is damaged	CONTROL -	Negative control
	Keep away from sunlight	CONTROL +	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@assaygenie.com with the technical data (if available) like standard curve, control results, etc. After the necessary examination, written reply will be given.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@ASSAYGenie.com

Web: www.ASSAYGenie.com