Technical Manual

Ustekinumab (Stelara®) ELISA Kit

- **Catalogue Code: HUMB00020**
- **Sandwich ELISA Kit**
- **Research Use Only**

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1. Intended Use

The Assay Genie Ustekinumab ELISA has been especially developed for the quantitative analysis of free ustekinumab in serum and plasma samples. Assay Genies' Ustekinumab ELISA is optimized with Stelara®.

2. Summary and Explanation

Ustekinumab is a human immunoglobulin (Ig) G1 kappa monoclonal antibody directed against interleukin (IL)-12 and IL-23. It was generated via recombinant human IL-12 immunization of human Ig (hu-Ig) transgenic mice. It is a targeted biologic disease-modifying anti-rheumatic drug (bDMARDs) that is used in the management of various inflammatory conditions that involve the activation of IL-12 and IL-23 signalling pathways.

Interleukin (IL)-12 and IL-23 are heterodimeric cytokines that evoke immune and inflammatory responses, such as natural killer cell activation and CD4+ T-cell differentiation and activation. The role of IL-12 and IL-23 were implicated in a variety of chronic inflammatory conditions, such as psoriasis and inflammatory bowel diseases. They modulate lymphocyte function, including T-helper (Th) 1 and Th17 cell subsets, as CD4+ T cells can differentiate into T-helper (Th) effector lineages based on the environment. Th cells can further activate the downstream pro-inflammatory mediators and transcription factors such as TNFα and IFNγ that drive innate and adaptive immunity.

IL-12 and IL-23 share a common p40 subunit, paired with p35 and p19 subunits of IL-12 and IL-23, respectively. The antigen-binding fragment (Fab) of ustekinumab binds the D1 domain of the p40 subunit of IL-12 and IL-23 in a 1:1 ratio. This prevents IL-12 and IL-23 from binding to the IL-12Rβ1 receptor chain of IL-12 (IL-12Rβ1/β2) and IL-23 (IL12Rβ1/23R) receptor complexes on the surface of NK and T cells. Ustekinumab only binds to IL-12 and IL-23 that are unbound to IL-12Rβ1, so it is unlikely to initiate Fc effector functions, such as ADCC or CDC. Inhibition of the IL-12/23 signalling pathway leads to profound suppression of both the Th1 and Th17 cell lineage of cytokines and chemokines and their inflammatory pathways.

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. Ustekinumab ELISA products:

Kit Name	Description	Product SKU
Ustekinumab (Stelara®) ELISA Kit	Free drug	HUMB00018
Anti- Ustekinumab (Stelara®) ADA Qualitative ELISA Kit	Antibody screening - Qualitative	HUMB00019

3. Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtiter plate coated with the reactant for ustekinumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to ustekinumab captured by the reactant 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 ustekinumab in the sample or standard. Results of samples can be determined directly using the standard curve.

4. Warnings and Precautions

1. For research 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. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.
7. Avoid contact with Stop solution. It may cause skin irritations and burns.
8. All reagents of this kit containing human serum or plasma (i.e. standards) have been tested and were found negative for HIV I/II, HBsAg and 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.
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.

5. 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 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.

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.
Standard AE	0.3 mL (each)	**Standards A-E (10X)** Standard A: 1000 ng/mL Standard B: 300 ng/mL Standard C: 100 ng/mL Standard D: 30 ng/mL Standard E: 0 ng/mL Used for standard curve and control. Contains ustekinumab, human serum, stabilizer and <0.1% NaN_3 Standards are prepared concentrated (10x). They should be diluted with the dilution rate given in the “Pre-test setup instructions” before the test.
Controls	0.3 mL (each)	**Control low and high levels (10x)** Contains human serum and stabilizer, <0.1% NaN_3 Controls are prepared concentrated (10x). They should be diluted with the dilution rate given in the “Pre-test setup instructions” before the test. Control concentrations are given in “Quality control certificate”
Assay Buffer	2 x 50 mL	**Assay Buffer** Blue coloured. Ready to use. Contains proteins and <0.1% NaN_3
Conjugate	1 x 12 mL	**Horse radish peroxidase-Conjugated Probe** Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives
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 of 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

1. 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.
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 Micro-pipettor 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.

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 37°C to dissolve crystals. Mix vigorously.
Storage	2-8°C
Stability	2 Weeks

- Dilution of standards, controls and samples

Sample	Standards and controls	Serum/Plasma
Diluent	Assay buffer	Assay buffer
Dilution Ratio	1/10	1/200
Remarks	1/10 dilution 20 µL standard/control + 180 µL assay buffer	For final dilution 1/200: − First step: 1/10 dilution 10 µL sample + 90 µL assay buffer − Second step: 1/20 dilution 20 µL sample (1/10 diluted) + 380 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

Total assay time: 70 minutes

Dilute each of the standards, controls and samples as described in “Pre-test Setup Instructions”

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Pipette 75 µL “Assay Buffer” into each of the wells to be used

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Pipette 25 µL of each diluted “Standards”, “Low level control”, “High level control” and diluted samples into the respective wells of microtiter plate

Wells

A1: Standard A
B1: Standard B
C1: Standard C
D1: Standard D
E1: Standard E
F1: Low level control
G1: High level control
H1 and on: Samples

↓

Cover the plate with adhesive foil
Briefly mix contents by gently shaking the plate
Incubate 30 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 “Conjugate” into each well

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Cover the plate with adhesive foil
Incubate 30 minutes at room temperature (18-25°C)

↓

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

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Incubate 10 minutes without adhesive foil at room temperature (18-25°C) in the dark

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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

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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. Calculation & Interpretation of Results

- Create a standard curve by using the standards. OD 450/650 nm for each standard on the vertical (Y-axis) axis versus the corresponding drug concentration on the horizontal (X-axis) axis.

− Standards and controls are prepared concentrated (10x). They should be diluted with the dilution rate given in the “Pre-test setup instructions” before the test. The standard curve should be prepared with the values obtained after dilution.

− 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 drug 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 drug concentration of the unknown sample.

− If computer data is going to be used, we recommend primarily "Four Parameter Logistic (4PL)" or secondly the "point-to-point calculation".

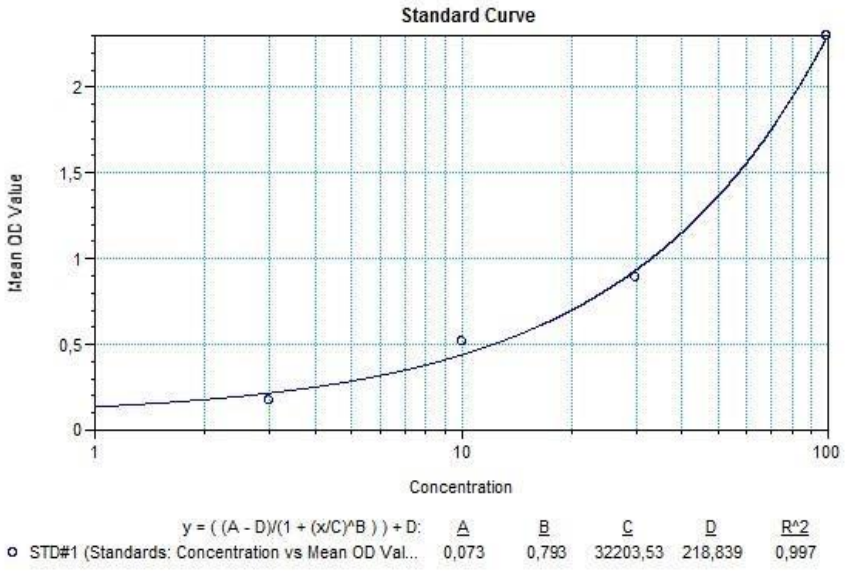
− To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (200x). Any sample reading greater than the highest standard should be further diluted appropriately with assay buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determined from the standard curve must be multiplied by the further dilution factor.

e.g.; If the pre-diluted sample further diluted in a ratio of 1/5 then results should be multiplied by 1000.

− For low and high level controls values, refer to “Quality Control Certificate” provided by each kit.

14. Typical Calibration Curve

− Calibration curve (Linearity, Dilutional linearity): r^2 >0.95



This is only an example. Assay conditions will change in every assay and do not use this curve for your assay calculations.

− Sensitivity: The lowest detectable level (Lowest detection limit, LOD) that can be distinguished from the zero standard is 2 ng/mL

Functional sensitivity (Limit of quantification-LOQ): 3 ng/mL

− Specificity: There is no cross reaction with native serum immunoglobulin
Recovery <100±30%.

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

− Reference range/Therapeutic range: There isn't any consensus for therapeutic ranges. Therapeutic ranges can be different for age, sex and diseases. Please refer to the latest literature for details.

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' Ustekinumab ELISA is also suitable to run on an automated ELISA processor.

16. Symbols and Cautions

	Manufacturer		Temperature limitation
	Production date		See instruction for use
	Expiry date		Caution
	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@assaygenie.com with the technical data (if available) like standard curve, control results, etc. After the necessary examination, written reply will be given.

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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