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ab264502
Ustekinumab ELISA Kit

For the measurement of Ustekinumab in human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Ustekinumab ELISA Kit (ab264502) is the only FDA-approved medicine that targets IL-12 and IL-23, which are thought to be associated with gastrointestinal inflammation in Crohn's disease. This kit is a sandwich-based ELISA kit. The color developed is proportional to the amount of Ustekinumab in the sample or standard. Results of samples can be determined directly using the standard curve.

Ustekinumab is a fully human monoclonal antibody that binds with high specificity and affinity to the cytokines interleukin (IL)-12 and IL-23, thereby suppressing IL-12- and IL-23-mediated inflammation associated with psoriasis.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 75 µL Assay Buffer to all wells.

Add 25 µL of Diluted Standards, High and Low Standards and diluted samples into the respective wells.

Cover with foil and incubate for 30 minutes at room temperature.

Add 100 µl HRP-conjugated Probe into all wells.

Cover with foil and incubate for 30 minutes at room temperature.

Discard the solution and wash plate 3 times with 300 µL diluted Wash Buffer

Add 100 µL TMB Substrate and incubate the plate in the dark at room temperature for 10 minutes. (No foil).

Add 100 µL Stop Solution and read OD at 450/650 nm within 30 minutes.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors.

6. Materials Supplied

Item	Quantity	Storage condition
Micro ELISA Plate	1 unit	+4°C
Ustekinumab Standard S1	200 µL	+4°C
Ustekinumab Standard S2	200 µL	+4°C
Ustekinumab Standard S3	200 µL	+4°C
Ustekinumab Standard S4	200 µL	+4°C
Ustekinumab Standard S5	200 µL	+4°C
Ustekinumab Standard S6	200 µL	+4°C
Ustekinumab Standard S7	200 µL	+4°C
Assay Buffer	2 x 50 mL	+4°C
HRP-conjugate Probe	12 mL	+4°C
TMB Substrate	12 mL	+4°C
Stop Solution	12 mL	+4°C
Wash Buffer (20X)	50 mL	+4°C
Plate sealers	2 units	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 20X Wash Buffer:

Dilute the 20X Wash Buffer to 1X solution in ddH_2O (10 mL of Wash Buffer stock to 190 mL of ddH_2O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

10. Standard and Control Preparation

1. First dilute standards at 1:10 (25 µL Standard + 225 µL Assay Buffer).
2. Diluted samples should further be diluted if the concentration of Ustekinumab is higher than the measuring range.

Name	S1	S2	S3	S4	S5	S6	S7
Stock Conc. ng/mL	1000	300	100	30	0	High Standard	Low Standard
Working Conc. ng/mL	100	30	10	3	0	Diluted High Standard	Diluted Low Standard

Concentration for high and low controls are indicated on vials.

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

11.1 Serum/plasma:

1. First dilute samples at 1:10 (10 µL serum/plasma + 90 µL Assay Buffer).
2. Then dilute samples 1:20 (20 µL diluted sample + 380 µL Assay Buffer).
3. Diluted samples should further be diluted if the concentration of Ustekinumab is higher than the measuring range.
4. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

Δ Note: The usual precautions for venipuncture should be observed.

12. Assay Procedure

- Prepare reagents within 30 minutes before the experiment.
- Equilibrate all materials and prepared reagents to room temperature 15 minutes prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1 Pipette 75µl of Assay Buffer non-exceptionally into each of the wells to be used.
12.2 Pipette 25 µL of each Diluted Standards, Diluted High Standard, Low Standard and Diluted Samples into the respective wells of microtiter plate.
12.3 Cover the plate with adhesive foil. Incubate 30 min at room temperature (18 - 25°C).
12.4 Remove adhesive foil. 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.
12.5 Pipette 100 µL of ready-to use HRP-Conjugated Probe into each well.
12.6 Cover the plate with adhesive foil. Incubate 30 min at room temperature (18- 25°C).
12.7 Remove adhesive foil. 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.
12.8 Pipette 100 µL of TMB Substrate Solution into each well.
12.9 Incubate 10 min (without adhesive foil.) at room temperature (18-25°C) in the dark.
12.10 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 yellow to blue
12.11 Measure optical density with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution.

13. Calculations

13.1 Calculate the average absorbance value for the blank control (0 ng/mL) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

13.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y axis) against the target protein concentration (x axis) of the standard.

13.3 Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to-point calculation curve fit.

13.4 To obtain the exact values of the samples, the concentration determined from the standard curve should be multiplied by the dilution factor.

13.5 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate dilution factor to obtain the concentration of target protein in the sample.

13.6 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

14. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

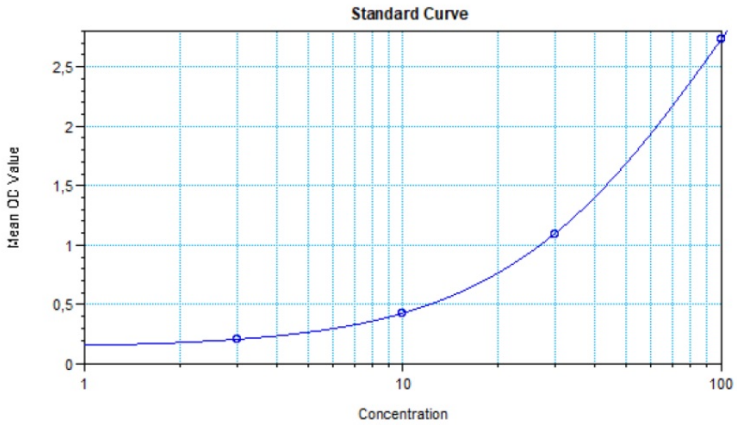

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

15. Typical Sample Values

Detection Range: 3 - 100 ng/mL.

Sensitivity: 3 ng/mL.

Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15%

(CV (%) = SD/mean X 100)

Cross Reactivity: No significant cross-reactivity or interference with other proteins present in native human serum or other therapeutic immunoglobulins.

Recovery rate: 85 – 115% with normal human serum samples with known concentrations.

16. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829