

Version 1a Last updated 12 April 2021

ab237667 anti-Ipilimumab ELISA Kit

For the measurement of the antibody against Ipilimumab 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

anti-Ipilimumab ELISA Kit (ab237667) is a highly specific and sensitive kit designed for the in vitro qualitative determination of antibody against Ipilimumab in biological matrices such as human serum and plasma.

Ipilimumab is a fully human IgG1 κ antibody that binds to CTLA-4 (cytotoxic T lymphocyte-associated antigen 4), a molecule on T cells that is indicated for unresectable or metastatic melanoma. The absence or presence of CTLA-4 can augment or suppress the immune system's T-cell response in fighting disease. Ipilimumab is designed to block the activity of CTLA-4, thereby sustaining an active immune response in its attack on cancer cells. The proposed mechanism of action is indirect, and may be through T-cell-mediated anti-tumor immune responses. However, some patients develop unwanted immunogenicity, which leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed. Add 100 μ L of Assay Buffer to each well.



Add 10 μ L of negative control, positive control and samples to appropriate wells. Cover and incubate for 60 minutes at room temperature



Discard incubation solution and wash plate 3 times with 300 μ L diluted Wash Buffer



Add 100 μ L peroxidase conjugate to each well. Cover and incubate for 60 minutes at room temperature



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



Add 100 μ L TMB Substrate and incubate the plate in the dark at room temperature for 20 minutes.



Add 100 μ L Stop Solution and read OD at 450 nm within 20 minutes.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- Reagents should be treated as possible mutagens and should be handled 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
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		condition
Micro ELISA Plate	1 unit	+4°C
Positive Control	0.25 mL	+4°C
Negative Control	0.5 mL	+4°C
Assay Buffer	12 mL	+4°C
Peroxidase Conjugate	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
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for sample dilution.
- Plate shaker for all incubation steps.
- Absorbent paper

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 ddH₂O (10 mL of Wash Buffer stock to 190 mL of ddH₂O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

10. Sample Preparation

General sample information:

- We recommend that you use fresh samples for the most reproducible assay.

10.1 Serum/plasma:

1. 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.

11. 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.
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- 11.1 Pipette 100 μ L of Assay Buffer into each of the wells to be used.
 - 11.2 Add 10 μ L negative control (2 wells), positive control, and samples into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
 - 11.3 Discard incubation solution. Wash plate 3 times each with 300 μ L of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
 - 11.4 Add 100 μ L of Peroxidase Conjugate into each well. Cover wells with adhesive plate sealer and incubate at room temperature for 60 minutes.
 - 11.5 Discard the solution and wash the wells as step 13.3.
 - 11.6 Add 100 μ L of 1X TMB substrate solution and incubate the plate in the dark at room temperature for 20 minutes.
 - 11.7 Add 100 μ L of Stop solution to stop the reaction.
 - 11.8 Read the absorbance in a microplate reader set to 450 nm within 20 minutes. (Reference wavelength to 650 nm).

12. Calculations

12.1 Qualitative Interpretation

- 12.1.1 For the run to be valid, the OD_{450/650} nm of Positive Control should be ≥ 1.000 and the OD_{450/650} nm of each Negative Control should be < 0.200 , if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- 12.1.2 If "Sample OD_{450/650} / Negative Control OD_{450/650}" is < 3 , the sample is NEGATIVE for Antibody to Ipilimumab.
- 12.1.3 If "Sample OD_{450/650} / Negative Control OD_{450/650}" is ≥ 3 , the sample is POSITIVE for Antibody to Ipilimumab.

13. Cross Reactivity

Cross Reactivity: Ipilimumab infusion camouflages/masks the presence of antibody to Ipilimumab (ATP) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATP. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Ipilimumab.

14. Notes

Technical Support

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