Version 2b Last updated 13 April 2021

ab237662 anti-Golimumab ELISA Kit

For the measurement of the antibody against Golimumab 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-Golimumab ELISA Kit (ab237662) is a highly specific and sensitive kit designed for the in vitro determination of the antibody against Golimumab in biological matrices such as human serum and plasma.

Golimumab is a human immunoglobulin G1κ monoclonal antibody which is specific for pro-inflammatory cytokine, tumor necrosis factor-a (TNFa). Elevated levels of TNF are found in the synovial fluid of rheumatoid arthritis, including juvenile idiopathic arthritis, psoriatic arthritis, and ankylosing spondylitis patients and play an important role in both the pathologic inflammation and the joint destruction that are hallmarks of these diseases. Golimumab binds to both the soluble and transmembrane bioactive forms of human TNF and prevent TNF from binding to its receptors and finally inhibits biological activity of TNF. 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 µL of Assay Buffer to each well.

Add 10 µL 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 µL diluted Wash Buffer

Add 100 µL peroxidase conjugate to each well. Cover and incubate for 60 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 20 minutes.

Add 100 µL 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 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
Positive Control	0.3 mL	+4°C
Negative Control	1 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 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. 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.

11.1 Pipette 100 µl of Assay Buffer into each of the wells to be used.
11.2 Add 10 µL 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 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
11.4 Add 100 µL 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 µL of 1X TMB substrate solution and incubate the plate in the dark at room temperature for 20 minutes.
11.7 Add 100 µL 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

- For the run to be valid, the OD 450/650 nm of positive control should be >1.5 and the OD 450/650 nm of each negative control should be <0.15. 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.
- If "Sample OD450/650 / Negative Control OD450/650" is < 3, the sample is NEGATIVE for Antibody to Golimumab.
- If "Sample OD450/650 / Negative Control OD450/650" is ≥ 3, the sample is POSITIVE for Antibody to Golimumab.

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.

13. Typical Sample Values

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

14. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)