Version 2 Last updated 27 June 2020

ab237646 Golimumab ELISA Kit (Simponi®)

For the measurement of Golimumab in human serum and plasma.

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

Table of Contents

1. Overview

Golimumab ELISA kit (ab237646) is a highly specific and sensitive kit designed for the in vitro determination of Golimumab in biological matrices such as human serum and plasma. The density of color is proportional to the amount of Golimumab captured from the samples and can be quantified when compared with standard curve.

Golimumab (Simponi®) is a human immunoglobulin G1κ monoclonal antibody which is specific for pro-inflammatory cytokine, tumor necrosis factor-a (TNFa). In 2009, it was approved by FDA for the treatment of rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis in adult patients. 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. Increased levels of TNF are also found in psoriasis (Ps) plaques. 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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 100 µL of Assay buffer to each well and add 10 µL of standard or sample to appropriate wells. Cover and incubate for 30 minutes at room temperature

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

Add 100 µL HRP-conjugate to each well. Cover 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.

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
Golimumab Standard S1	300 µL	+4°C
Golimumab Standard S2	300 µL	+4°C
Golimumab Standard S3	300 µL	+4°C
Golimumab Standard S4	300 µL	+4°C
Golimumab Standard S5	300 µL	+4°C
Golimumab Standard S6	300 µL	+4°C
Golimumab Standard S7	300 µL	+4°C
Assay Buffer	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
- 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. Standard and Control Preparation

Standard and controls, S1 – S7, are ready to use, please see table below for concentrations:

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (µg/ml)	3	1	1.3	0.3	0.1	High control	Low control

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:

11.1.1 Dilute samples at 1:10 (20 µL serum/plasma + 180 µL Assay buffer).

11.1.2 Diluted samples should further be diluted if the concentration of Golimumab is higher than the measuring range.

11.1.3 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 Add 100 µL of Assay buffer to each well.

12.2 Add 10 µL of standards, controls and diluted samples into appropriate wells. Cover wells and incubate for 30 minutes at room temperature.

12.3 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.

12.4 Add 100 µL of HRP-conjugate into each well. Cover wells with adhesive plate sealer and incubate at room temperature for 30 minutes.

12.5 Discard the solution and wash the wells as step 12.3.

12.6 Add 100 µL of 1X TMB substrate solution and incubate the plate in the dark at room temperature for 10 minutes.

12.7 Add 100 µL of Stop solution to stop the reaction.

12.8 Read the absorbance in a microplate reader set to 450 nm within 20 minutes. (Reference wavelength to 650 nm).

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.

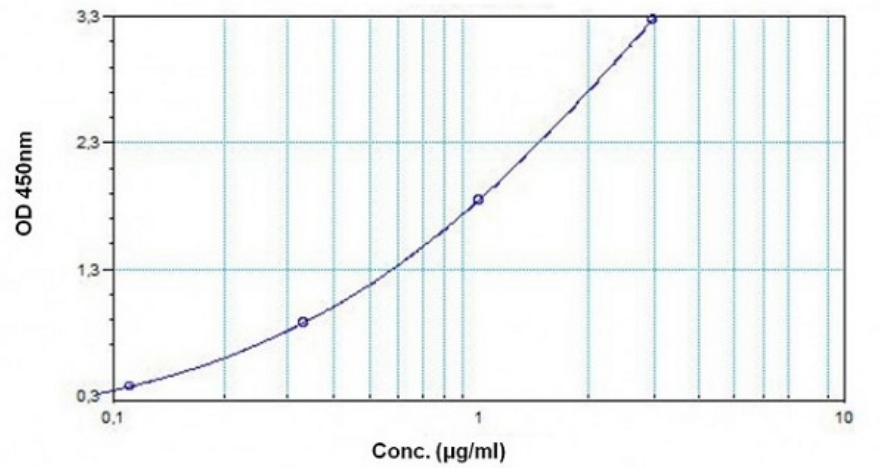

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

15. Typical Sample Values

Detection Range: 100 - 3000 ng/mL.

Sensitivity: 100 ng/mL.

Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (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: <100±30% 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