Instruction for Use
Golimumab (Simponi®) ELISA

SHIKARI® Q-GOL

Enzyme immunoassay for the quantitative determination of golimumab (Simponi®) in serum, plasma

REF TR-GOLv1 \[ \sum \] 12 x8

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Matriks Biotek® Laboratories
www.matriksbiotek.com
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<tbody>
<tr>
<td>Free Golimub (Simponi®) quantitative analyses</td>
</tr>
<tr>
<td>Required Volume (µl)</td>
</tr>
<tr>
<td>Total Time (min)</td>
</tr>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>Sample Number</td>
</tr>
<tr>
<td>Detection Limit (ng/mL)</td>
</tr>
<tr>
<td>Spike Recovery (%)</td>
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<tr>
<td>Shelf Life (year)</td>
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</table>
Intended Use

Enzyme immunoassay for the quantitative determination of free golimumab in serum and plasma. Matriks Biotek® golimumab ELISA has been especially developed for the quantitative analysis of free golimumab in serum and plasma samples.

Summary and Explanation

Golimumab (Simponi, CNTO-148) is a human immunoglobulin G1κ monoclonal antibody which is specific for pro-inflammatory cytokine, tumor necrosis factor-α (TNFα). In 2009, it was approved by FDA for the treatment of rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis in adult patients. TNF is a naturally occurring cytokine that is involved in normal inflammatory and immune responses. 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. Its affinity for TNF in surface plasmon resonance assay was 17 pmol/L. Golimumab was approved to be used in the underlined diseases alone or combination with methotrexate (MTX). According to American College of Rheumatology (ACR) criteria, golimumab 50 or 100 mg every 4 weeks in combination with MTX in MTX-naïve (GO-BEFORE) and MTX-experienced (GO-FORWARD) rheumatoid arthritis patients was more effective than MTX alone to overcome the symptoms at week 14 and/or 24. In rheumatoid arthritis treated with other anti-TNF agents (GO-AFTER) before, golimumab 50 or 100 mg every 4 weeks was more effective than placebo at week 14 and/or 24. These affirmative influences were also shown for psoriatic arthritis GO-REVEAL and ankylosing spondylitis GO-RAISE studies.
Pharmacokinetics and Pharmacodynamics

With golimumab 100 mg every 2 or 4 weeks and 50 mg every 4 weeks, median C-reactive protein (CRP) level in rheumatoid arthritis patients decreased to normal at week 2. In patients with rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis, golimumab was shown to reduce serum levels of inflammatory biomarkers that corresponded to clinical benefits.

In patients with rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis, absorption constant values of golimumab was determined as 0.668, 0.908 and 1.01 per day, respectively. Estimated bioavailability of subcutaneous golimumab was 53%.

In patients with rheumatoid arthritis who were administered by 50 or 100 mg every 2 or 4 weeks, serum golimumab steady-state ($C_{SS}$) was attained by week 12. Median peak $C_{SS}$ values 3 days after the week 16 injection of golimumab 50 mg every 4 weeks, golimumab 50 mg every 2 weeks, golimumab 100 mg every 4 weeks, and golimumab 100 mg every 2 weeks were 1.7, 3.8, 4.1, and 7.8 ug/mL, respectively. Median trough $C_{SS}$ concentrations were 0.5, 1.2, 1.2, and 3.4 ug/mL, respectively.

The apparent volume of distribution for golimumab in a typical 70 kg patient was 26.7L in methotrexate-naive patients with rheumatoid arthritis, 24.9 L in patients with psoriatic arthritis, and 22.6 L in patients with ankylosing spondylitis.

The apparent clearance rate of golimumab was 1.91 L/day in patients with rheumatoid arthritis, 1.38 L/day in patients with psoriatic arthritis, and 1.41 L/day in patients with ankylosing spondylitis.

Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and diluted samples (serum or plasma) are incubated in the microtitre plate coated with the reactant for golimumab (Simponi®). After incubation, the wells are washed. A horse radish peroxidase (HRP) conjugated probe is added and binds to golimumab (Simponi®) 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 chromogen-substrate. The colour developed is proportional to the amount of golimumab in the sample or standard. Results of samples can be determined directly using the standard curve.
Warnings and Precautions

1. For professional 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. In case of severe damage of the kit package please contact Matriksbiotek or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.

4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.

7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.

8. Avoid contact with Stop solution. It may cause skin irritations and burns.

9. Some reagents contain sodium azide (NaN₃) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN₃ 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.

10. All reagents of this test kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV by FDA approved procedures. 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.
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 storage and stability of specimen and prepared reagents is stated in the corresponding chapters. 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 or -20°C.

Specimen Collection and Storage

Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

<table>
<thead>
<tr>
<th>Storage:</th>
<th>2-8°C</th>
<th>-20°C</th>
<th>Keep away from heat or direct sun light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability:</td>
<td>7 d</td>
<td>6 mon</td>
<td>Avoid repeated freeze-thaw cycles</td>
</tr>
</tbody>
</table>

* Golimumab (Simponi®) infusion camouflages/masks the presence of antibody to golimumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of golimumab. Matriks Biotek® Laboratories propose to obtain blood sample just before the infusion of golimumab (Simponi®) or at least 2 weeks after the infusion of golimumab (Simponi®).
## Materials Supplied

<table>
<thead>
<tr>
<th>Item Code</th>
<th>Description</th>
<th>Specification</th>
</tr>
</thead>
</table>
| 1 x 12 x 8 | MTP | **Microtiter Plate**  
Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant. |
| 7 x 0.3 mL | STND A-E HIGH CNTRL LOW CNTRL | **Golimumab Standards A-E, High Level Control, Low Level Control**  
3000; 1000; 300; 100; 0 ng/mL Ready to use. Used for construction of the standard curve. Contains golimumab (Simponi®), human serum, stabilizer and <0.1 % NaN₃ |
| 1 x 50 mL | ASSAY BUF | **Assay Buffer**  
Blue colored. Ready to use. Contains proteins, and <0.1% NaN3 |
| 1 x 12 mL | HRP CONJ | **Peroxidase Conjugate**  
Red colored. Ready to use. Contains peroxidase (POD) conjugate, stabilizer and <0,1% NaN3. |
| 1 x 12 mL | TMB SUBS | **TMB Substrate Solution**  
Ready to use. Contains TMB |
| 1 x 12 mL | TMB STOP | **TMB Stop Solution**  
Ready to use. 1N HCl. |
| 1 x 50 mL | WASHBUF CONC | **Wash Buffer, Concentrate (20x)**  
Contains Buffer with Tween 20. |
| 2 x 1 | ADH FILM | **Adhesive Film**  
For covering of Microtiter Plate during incubation. |
| 2 x 1 | SLGP | **Semi Log Graph Paper**  
For constructing standard curve and calculation of results. |
Materials Required but not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Calibrated measures.
3. Tubes (1 mL) for sample dilution.
4. Wash bottle, automated or semi-automated microtiter plate washing system.
5. Microtiter plate reader capable of reading absorbance at 450/650 nm.
6. Bidistilled or deionised water, paper towels, pipette tips and timer.

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 pretreatment steps have to 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 Micropipettor 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.
1. Preparation of Components

<table>
<thead>
<tr>
<th>Dilute/ dissolve</th>
<th>Component</th>
<th>with</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL</td>
<td>Wash Buffer*</td>
<td>Up to 200 mL</td>
<td>Bidist. water</td>
<td>1:20</td>
<td>Warm up at 37°C to dissolve crystals. Mix vigorously.</td>
<td>2-8 °C</td>
<td>2 w</td>
</tr>
</tbody>
</table>

*. Prepare Wash Buffer before starting assay procedure.

2. Dilution of Samples (serum/plasma)

<table>
<thead>
<tr>
<th>Sample</th>
<th>To be diluted</th>
<th>With</th>
<th>Relation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/ Plasma</td>
<td>Initially 1:10</td>
<td>Assay Buffer</td>
<td>1:10</td>
<td>For dilution at 1:10; 20µL Sample + 180µL Assay Buffer</td>
</tr>
</tbody>
</table>

Patient samples with a concentration of golimumab 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.
## Test Procedure

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used</td>
</tr>
</tbody>
</table>
|2| Pipette 10 µL of **Ready-to-use Standards, High Level Control, Low 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: High Level Control  
G1: Low Level Control  
H1 and on: Sample (Serum / Plasma) |
|3| Cover the plate with adhesive film. Briefly mix contents by gently shaking the plate. Incubate **30 min** at room temperature (18-25°C). |
|4| Remove adhesive film. 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. |
|5| Pipette 100 µL of ready-to-use HRP Conjugate into each well. |
|6| Cover the plate with adhesive film. Incubate **30 min** at room temperature (18-25°C). |
|7| Remove adhesive film. 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. |
|8| Pipette 100 µL of TMB Substrate Solution into each well. |
|9| Incubate **10 min** (without adhesive foil.) at room temperature (18-25°C) in the dark. |
|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 blue to yellow |
|11| Measure optical density with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution. |
Quality Control

The test results are only valid only 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. All standards must be found within the acceptable ranges as stated above and/or label. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation & Interpretation of Results

1. Using the standards (3000; 1000; 300; 100; 0 ng/mL) disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each of 4 standards on the vertical (Y-axis) axis versus the corresponding golimumab concentration on the horizontal (X-axis) axis, thus creating a standard curve by 4 points obtained.

2. 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 golimumab 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 golimumab concentration for the unknown sample.

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

4. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (10x). 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 1:10 then results should be multiplied by 100.**

5. Automated method: Computer programs can also generally give a good fit.
Typical Calibration Curve
(Example. Do not use for calculation!)

\[
y = \frac{(A - D)(1 + (x/C)x)}{B} + D
\]

<table>
<thead>
<tr>
<th>Standart</th>
<th>Concentration (ng/mL)</th>
<th>Mean OD450/650</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3000</td>
<td>3.264</td>
</tr>
<tr>
<td>B</td>
<td>1000</td>
<td>1.835</td>
</tr>
<tr>
<td>C</td>
<td>300</td>
<td>0.865</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>0.359</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0.054</td>
</tr>
</tbody>
</table>
**Assay Characteristics**

1. **Specificity:** Except for the other therapeutic anti-TNF antibodies such as etanercept (Enbrel®) and adalimumab (Humira®) with which cross reaction might occur to some extends, there is no cross reaction with native serum immunoglobulin.

2. **Sensitivity:** The lowest detectable level that can be distinguished from the zero standard is 10 ng/mL.

3. **Precision Of Kit:**
   - **Intra-assay CV:** <15% for golimumab range 3000-100 ng/mL.
   - **Inter-assay CV:** <15% for golimumab range 3000-100 ng/mL.

4. **Recovery:** Recovery rate was found to be between 85-115% with normal human serum samples with known concentrations.

**Automation**

Experiments have shown that the *Matriks Biotek® SHIKARI® Golimumab ELISA* is also suitable to run on an automated ELISA processor.
References


Golimumab Concentration (ng/ml)