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| | SHIKARI Q-GOL |
|---|---|
| | Golimumab (Simponi® ) quantative analyse |
| Required Volume (µl) | 10 |
| Total Time (min) | 70 |
| Sample | Serum, plazma and other biological fluids |
| Sample Number | 96 |
| Detection Limit µg/mL) | 0,1 |
| Spike Recovery (%) | 93 |
| Shelf Life (year) | 1 |

## 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**

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.

# 1. Preparation of Components

| Dilute/ disolve | Component | with | Diluent | Rela-tion | Remarks | Storage | Stability |
|---|---|---|---|---|---|---|---|
| 10 mL | Wash Buffer* | Up to 200 mL | Bidist. water | 1:20 | Warm up at 37°C to dissolve crystals. Mix vigorously. | 2-8 °C | 3 w |

*. Prepare Wash Buffer before starting assay procedure.

# 2. Dilution of Standards and Samples (serum/plasma)*

| Sample | To be diluted | With | Remarks |
|---|---|---|---|
| 10 µL | 1:10 | Assay Buffer | For dilution at 1:10;<br>10µl Serum or Sample + 90µl Assay Buffer |

*. In case any patient samples with a concentration of golimumab (Simponi®) above the measuring range are to be rated as "> highest standard". The result must not be extrapolated. The patient sample in question should be further diluted with Assay Buffer and retested.

# Test Procedure

| | |
|---|---|
| 1 | Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used |
| 2 | Pipette 10 µL of Z[redacted] Standards and Samples into the respective wells of microtiter plate.<br>**Wells**<br>A1: S[redacted]tandard A<br>B1: S[redacted]tan dard B<br>C1: Standard C<br>D1: Standard D<br>E1: Standard E<br>F1: 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 o 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.

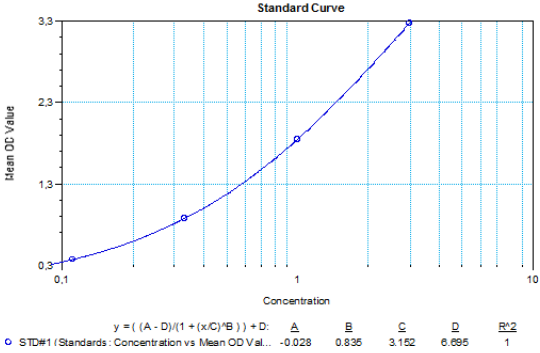
## Calculation & Interpretation of Results

1. A standard curve should be calculated using the standard concentration (X-axis) versus the OD450 (we suggest that OD readings done at double wavelength at OD450/650 and OD value read at 650nm subtracted from 450nm OD VALUE) VALUES (Y-axis). In case of manual plot, we suggest semilog graph paper. If computer data regation is going to be used, we recommend primarily "4 Parameter Logistic (4PL)" or secondly the "point-to-point calculation".

2. The concentration of the samples can be read from this standard curve as follows. Using the absorbance value for each sample, determine the corresponding concentration of the drug from standard curve. This value always has to be multiplied by the dilution factor. If any diluted sample is reading greater than highest standard, it should be further diluted appropriately with Assay Buffer and retested. Also this second dilution has to be used for calculation the final results.

3. Any sample diluted at 1:10 and still reading greater than the highest standard should be further diluted appropriately with Assay Buffer and retested. **Because the samples have been diluted, the concentration determined from the standardcurve must be multiplied by the dilution factor.**

**Typical Calibration Curve**
(Example. Do not use for calculation!)




| Standart | Concentration (µg/mL) | Mean OD450/650 |
|---|---|---|
| A | 3 | 2,711 |
| B | 1 | 1,817 |
| C | 0,3 | 1,069 |
| D | 0,1 | 0,462 |
| E | 0 | 0,049 |

## Assay Characteristics

**1. Specificity:** Except for the other therapeutic anti-TNF antibodies such as etanercept (Enbrel®) and/or 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 0,1μg/mL.

**3. Precision Of Kit:**
**Intra-assay CV:** <20% for golimumab range 0.1-3 ug/mL.
**Inter-assay CV:** <20% for golimumab range 0.1-3 ug/mL.

**4. Recovery:** Recovery rate was found to be equal and higher than 98% with normal human serum samples with known concentrations.

## Automation

Experiments have shown that the golimumab ELISA is also suitable to run on an automated ELISA processor.

# References


1. Kay J, Matteson EL, Dasgupta B, et al. Golimumab in patients with active rheumatoid arthritis despite treatment with methotrexate: a randomized, double-blind, placebo-controlled, dose-ranging study. Arthritis Rheum 2008 Apr; 58 (4): 964-75.

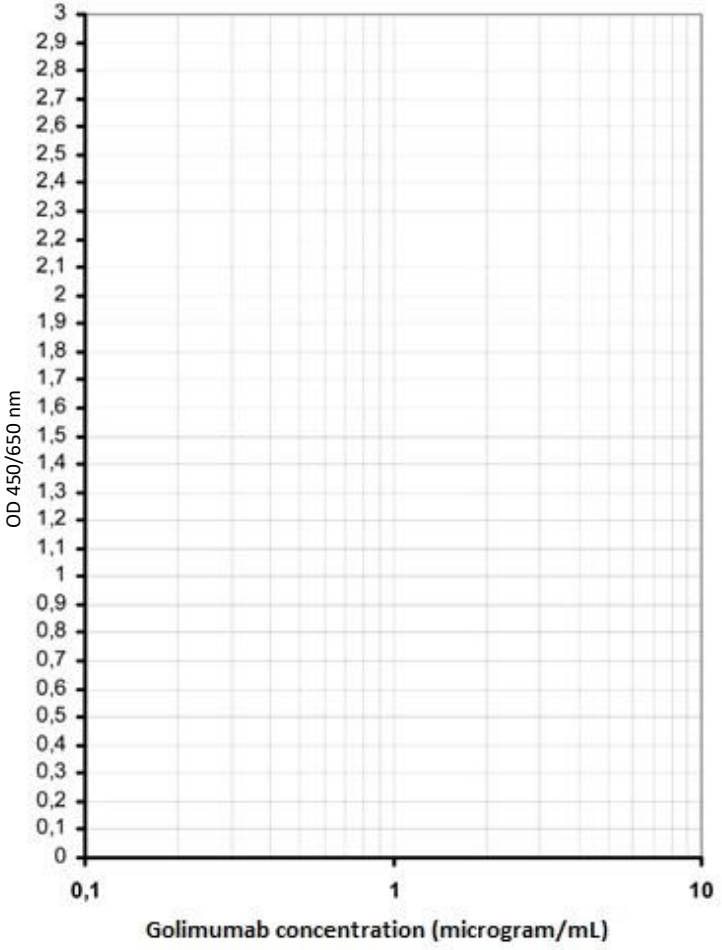
2. Pappas et al. 2009. Golimumab. Nature Reviews Drug Discovery 8 695-696.

3. Shealy D, Cai A, Lacy E, et al. Characterization of golimumab (CNTO 148), a novel fully human monoclonal antibody specific for human TNFalpha [abstract no. THU0088]. 2007 Annual European Congress of Rheumatology; 2007 Jun 13-16; Barcelona.

4. Xu Z, Lee H, Vu T, et al. Population pharmacokinetics of golimumab, an antitumor necrosis factor-alpha human monoclonal antibody, in patients with rheumatoid arthritis [abstract no. FRI0149]. 2008 Annual European Congress of Rheumatology; 2008 Jun 11-14; Paris.

5. Xu Z, Vu T, Lee H, et al. Population pharmacokinetics of golimumab, an anti-tumor necrosis factor-alpha human monoclonal antibody in patients with psoriatic arthritis [abstract no. SAT0316]. 2008 Annual European Congress of Rheumatology; 2008 Jun 11-14; Paris.

6. Xu Z, Vu T, Lee H, et al. Population pharmacokinetics of golimumab, an anti-tumor necrosis factor-alpha human monoclonal antibody in patients with ankylosing spondylitis [abstract no. AB0675]. 2008 Annual European Congress of Rheumatology; 2008 Jun 11-14; Paris.


OD 450/650 nm
3
2,9
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2,7
2,6
2,5
2,4
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2,2
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2
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1
0,9
0,8
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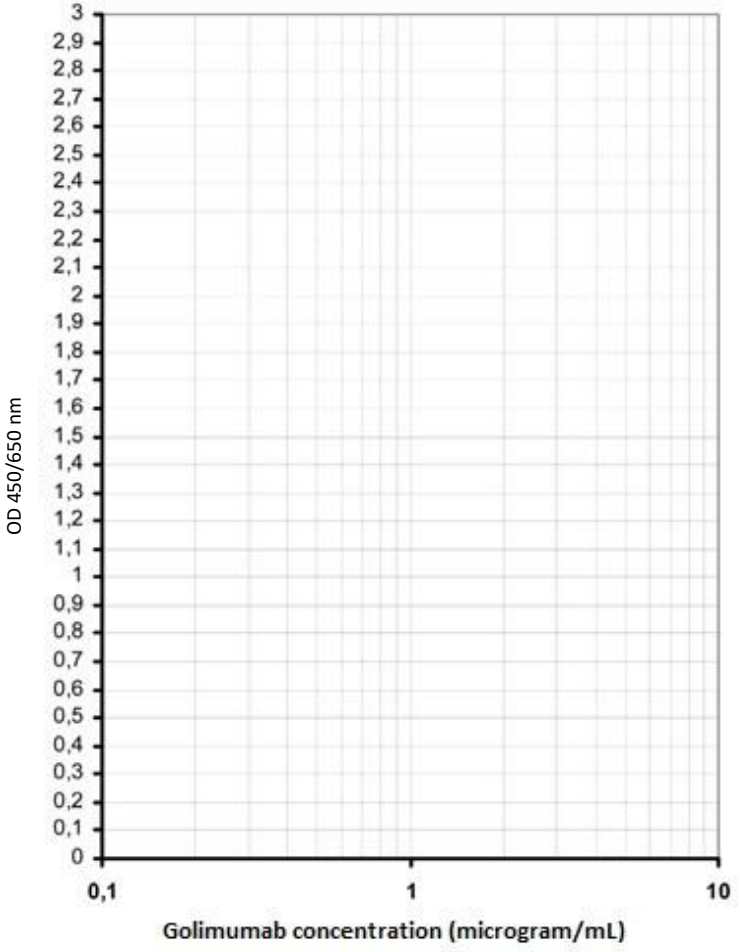
0,6
0,5
0,4
0,3
0,2
0,1
0
0,1
1
10
Golimumab concentration (microgram/mL)


OD 450/650 nm
3
2,9
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1,9
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Golimumab concentration (microgram/mL)