MATRIKS BIOTEK
innovation for health & wellness

"trace & catch"

Instructions for Use

Certolizumab pegol (Cimzia®) ELISA

SHIKARI®
Q-CERT

Enzyme immunoassay for the quantitative determination of certolizumab pegol (Cimzia®) in serum and plasma

REF **TR-CERTv1** 12 x 8 2-8 C

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Matriks Biotek® Laboratories
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	Free certolizumab pegol (Cimzia®) quantitative analyses
Required Volume (µl)	10
Total Time (min)	70
Sample	Serum, plasma
Sample Number	96
Detection Limit (ng/mL)	12
Spike Recovery (%)	Between 85-115
Shelf Life (year)	1

Intended Use

Enzyme immunoassay for the quantitative determination of **free certolizumab pegol** (Cimzia®) in serum and plasma. *Matriks Biotek® Certolizumab pegol ELISA* has been especially developed for the quantitative analysis of free certolizumab pegol in serum and plasma samples at high specificity.

Summary and Explanation

Certolizumab pegol is an anti-TNF (anti-tumour necrosis factor) drug. It blocks the action of TNF protein and so reduces inflammation in conditions including rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis.

TNFα is a key pro-inflammatory cytokine with a central role in inflammatory processes. Biological activity associated to TNFα include the upregulation of cellular adhesion molecules and chemokines, upregulation of major histocompatibility complex (MHC) class I and class II molecules, and direct leukocyte activation. TNFα stimulates the production of downstream inflammatory mediators, including interleukin-1, prostaglandins, platelet activating factor, and nitric oxide. After treatment with certolizumab pegol, patients with Crohn's disease demonstrated a decrease in the levels of C-reactive protein (CRP).

Certolizumab pegol binds to free and membrane-bound human TNFα with a KD of 90pM and neutralizes its activity. Extent of neutralization is also dose-dependent. It also inhibited the release of lipopolysaccharide-induced IL-1β from monocytes. TNFα is a key pro-inflammatory cytokine with a central role in inflammatory processes in which elevated levels have been observed in patients with RA and Crohn's. Certolizumab pegol selectively neutralizes TNFα. It does not bind to TNF-β. As certolizumab is only a Fab' fragment and thus missing the Fc region, it does not fix complement or cause antibody-dependent cell-mediated cytotoxicity. Furthermore, apoptosis of monocytes or lymphocytes, or neutrophil degranulation have not been observed in vitro.

There is a linear relationship between dose administered and Cmax and AUC. A mean Cmax of approximately 43 to 49 mcg/mL occurred at Week 5 during the initial loading dose period using the recommended dose regimen for the treatment of patients with rheumatoid arthritis (400 mg sc at Weeks 0, 2 and 4 followed by 200 mg every other week). Tmax, SubQ dose = 54 - 171 hours; Bioavailability, SubQ dose = 80% (range of 76% - 88%)

Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtitre plate coated with the reactant for certolizumab pegol (Cimzia®). After incubation, the wells are washed. A horse radish peroxidase (HRP) conjugated probe is added and binds to certolizumab pegol 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 certolizumab pegol 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 un-derstood. 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 Matriks Biotek® 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. All reagents of this kit containing human serum or plasma (i.e. standards) have been tested and were found negative for HIV I/II, HBsAg and HCV. 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.
10. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 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.

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 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.

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.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles
Stability:	2 d	6 mon	

*. Certolizumab pegol (Cimzia®) infusion camouflages/masks the presence of antibody to certolizumab pegol (ATC) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATC. The Matriks Biotek® Laboratories suggests to obtain blood sample just before the infusion of certolizumab pegol (Cimzia®) or at least 2 weeks after the infusion of certolizumab pegol (Cimzia®)

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant.
8 x 0.5 mL	STND A-F HIGH CNTRL LOW CNTRL	**Certolizumab pegol Standards A-F, High Level Control, Low Level Control** 1000; 333; 111; 37;12; 0 nanogram/mL Ready to use. Used for construction of the standard curve. Contains certolizumab pegol (Cimzia®), human serum, stabilizer and <0.1% NaN_3.
1 x 50 mL	ASSAY BUF	**Assay Buffer** Blue colored. Ready to use. Contains proteins and <0.1% NaN_3.
1 x 12 mL	HRP CONJ	**Horse radish peroxidase-Conjugated Probe** Red colored. Ready to use. Contains HRP-probe, stabilizer and preservatives.
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	FOIL	**Adhesive Foil** For covering of Microtiter Plate during incubation.
2x 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 interrup-tion. 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 plas-tic 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 re-sealed pouch including the desiccant.

Pre-Test Setup Instructions

1. Preparation of Components

Dilute/ disolve	Component	with	Diluent	Relation	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	2 w

***.** Prepare Wash Buffer before starting assay procedure.

2. Dilution of Samples

Sample	To be diluted	With	Remarks
Serum/ Plasma	1:50	Assay Buffer	For dilution at 1:50; 10 µl Sample + 490 µl Assay Buffer

Patient samples with a concentration of certolizumab pegol 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

1	Pipette 50µl of Assay Buffer non-exceptionally into each of the wells to be used.
2	Pipette 50 µL of each **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: Standard F G1: High Level Control H1: Low Level Control A2 and on:Sample (Serum / Plasma)
3	Cover the plate with adhesive foil. Incubate **30 min** at room temperature (18-25°C).
4	Remove adhesive foil. 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-Conjugated Probe into each well.
6	Cover the plate with adhesive foil. Incubate **30 min** at room temperature (18-25°C).
7	Remove adhesive foil. 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.

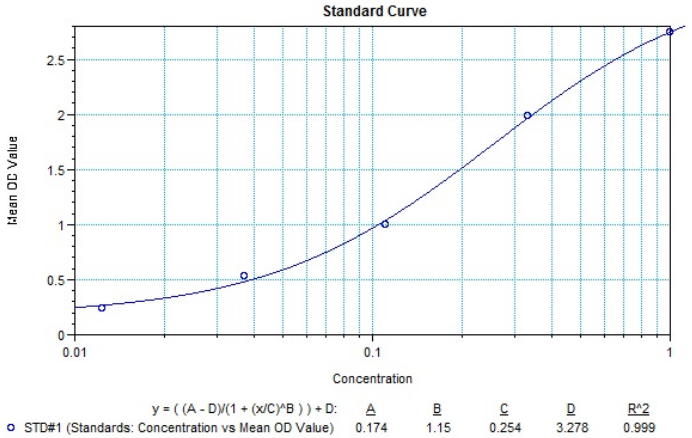
Calculation & Interpretation of Results

1. Using the standards (1000; 333; 111; 37; 12; 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 certolizumab pegol 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 certolizumab pegol 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 certolizumab pegol 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 (50x). 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 determind 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 1:5 then results should be multiplied by 250.*
5. Automated method: Computer programs can also generally give a good fit.
6. For the OD values of **High Level** and **Low Level** controls, please reffer to **Quality Control Certificate** (QCC) provided by each kit

Typical Calibration Curve

(Example. Do not use for calculation!)



Standard	Concentration (ng/mL)	Mean OD450/650nm
A	1000	2,701
B	333	1,977
C	111	1,006
D	37	0,515
E	12	0,235
F	0	0,171

Assay Characteristics

1. **Specificity:** Except for certolizumab pegol, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.
2. **Sensitivity:** The lowest detectable level that can be distinguished from the zero standard is 12 ng/mL.
3. **Precision Of Kit:**
   Intra-assay CV: <15% for certolizumab pegol range 12-1000 ng/mL.
   Inter-assay CV: <15% for certolizumab pegol range 12-1000 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® Certolizumab pegol ELISA is also suitable to run on an automated ELISA processor.

References

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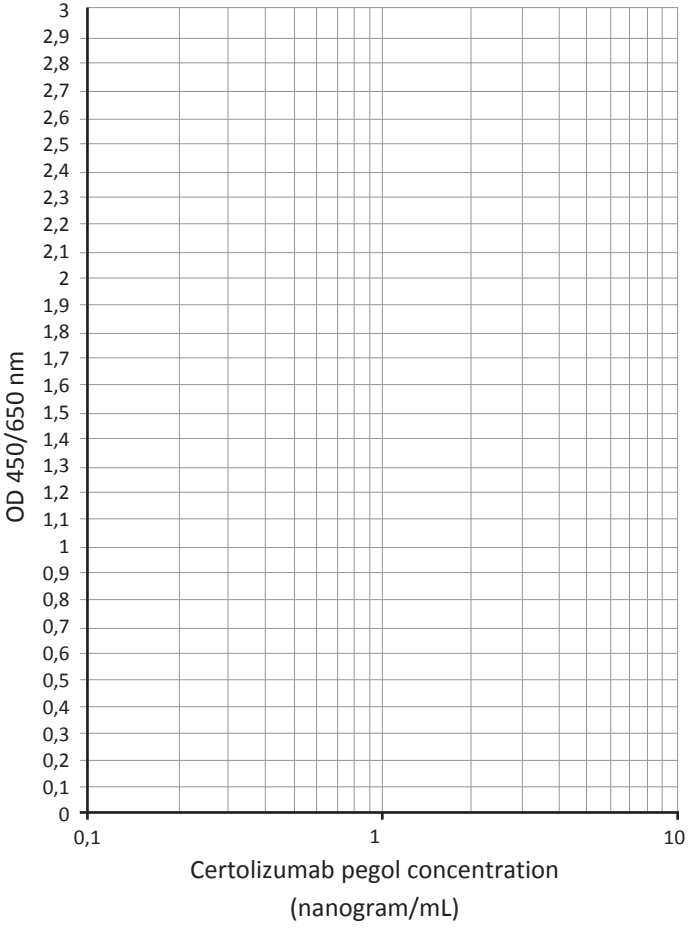
OD 450/650 nm
3
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2,5
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2,2
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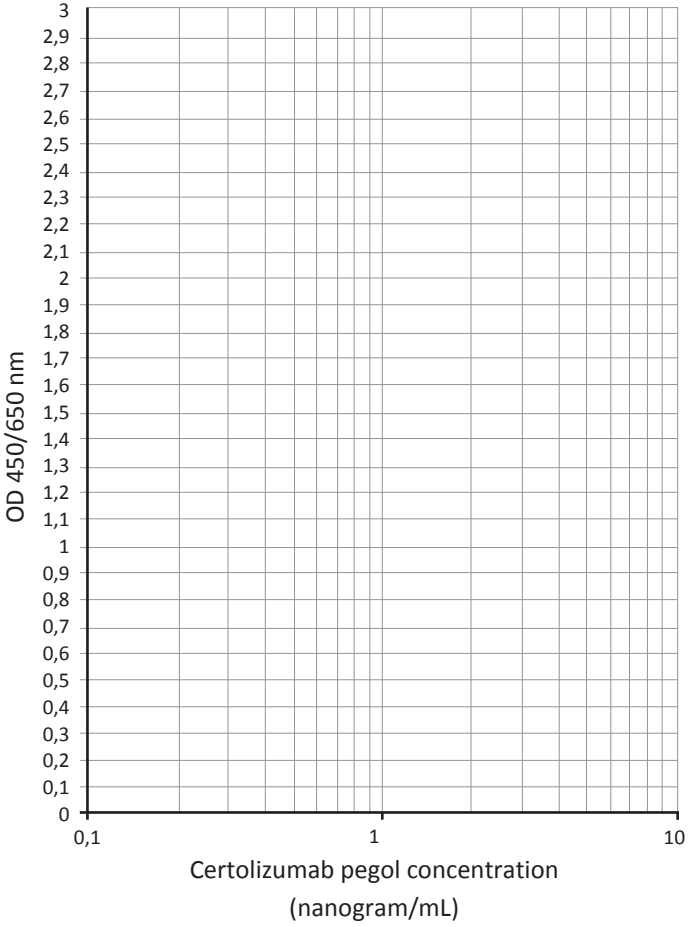
0,3
0,2
0,1
0
0,1
1
10
Certolizumab pegol concentration
(nanogram/mL)


OD 450/650 nm
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0,1
0
0,1
1
10
Certolizumab pegol concentration
(nanogram/mL)