MATRIKS BIOTEK

"trace & catch"

Instructions for Use

SHIKARI® (Q-CAN)

Canakinumab ELISA

Enzyme immunoassay for the quantitative analysis of free Canakinumab in serum and plasma sample

REF	**CAN-FD-ILA**
Σ	96 tests
	Shipment 10-30°C, Store 2-8°C
	MATRIKS BIOTECHNOLOGY CO., LTD. Bahcelievler Mah. 323/1 Cad. Gazi Universitesi Teknokent Binası C Blok No:10/50C/47 06830 Golbasi Ankara / TURKEY Tel +90 312 485 42 94 info@matriksbiotek.com
CE IVD	

1. Intended Use

SHIKARI® Canakinumab ELISA has been especially developed for the quantitative analysis of free Canakinumab in serum and plasma samples. SHIKARI® Canakinumab ELISA is optimized with Ilaris®.

2. General Information

Canakinumab is a recombinant, human anti-human-IL-1ß monoclonal antibody that belongs to the IgG1/κ isotype subclass. Canakinumab binds to human IL-1ß and neutralizes its inflammatory activity by blocking its interaction with IL-1 receptors, but it does not bind IL-1alpha or IL-1 receptor antagonist (IL-1ra).

In inflammatory diseases involving Cryopyrin-Associated Periodic Syndromes (CAPS), interleukin-1 beta (IL-1ß) is excessively activated and drives inflammation. The protein cryopyrin controls the activation of IL-1ß, and mutations in cryopyrin's gene, NLRP-3, up-regulate IL-1ß activation. Canakinumab is a human monoclonal anti-human IL-1ß antibody of the IgG1/κ isotype. Canakinumab binds to human IL-1ß and neutralizes its inflammatory activity by blocking its interaction with IL-1 receptors, but it does not bind IL-1α or IL-1 receptor antagonist (IL-1ra).

SHIKARI® ELISA kits can be used for drug level and anti-drug antibodies measurements. SHIKARI® Canakinumab ELISA products:

Brand	Description		Product Code
SHIKARI® (Q-CAN)	Canakinumab	Free Drug	**CAN-FD-ILA**
SHIKARI® (S-ATCAN)	Canakinumab	Antibody screening - Qualitative	**CAN-QLS-ILA**
SHIKARI® (S-ATCAN)	Canakinumab	Antibody screening - Quantitative	**CAN-QNS-ILA**

Check the web page for the whole product list www.matriksbiotek.com

3. Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtiter plate coated with the reactant for canakinumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to canakinumab 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 tetramethylbenzidine (TMB) chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed is proportional to the amount of canakinumab in the sample or standard. Results of samples can be determined directly using the standard curve.

4. Warnings and Precautions

- For professional use only.

- 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.

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

- 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.

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

- All reagents of this kit containing human serum or plasma (standards etc.) have been tested and were found negative for HIV I/II, HBsAg and Anti-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.

- Reagents of this kit containing hazardous material may cause eye and skin irritations. See "Materials supplied", SDS and labels for details.

- Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.

5. Storage and Stability

The kit is shipped at ambient temperature (10-30°C) and should be stored at 2-8°C for long term storage. Keep away from heat or direct sunlight. The strips of microtiter plate are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

6. Specimen (Collection and Storage)

Serum, Plasma (EDTA, Heparin)

The usual precautions for venipuncture should be observed. Do not use grossly haemolytic, icteric or lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Avoid repeated freeze- thaw cycles for serum/plasma samples.

Samples should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test.

Drug infusions may camouflages/mask the presence of antibody to drugs in serum/plasma samples. Therefore, blood sampling time is critical for detection of antibodies. It is recommended to take the blood sample just before the scheduled dose (trough specimen).

Storage	2-8°C	-20°C
Stability (serum/plasma)	2 days	6 months

7. Materials Supplied

Microtiter Plate	1 x 12 x 8	**Microtiter plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant.
Standard A-E	0,3 mL (each)	**Standards A-E (10X)** Standard A: 1000 ng/mL Standard B: 300 ng/mL Standard C: 100 ng/mL Standard D: 30 ng/mL Standard E: 0 ng/mL Ready to use. Used for the standard curve and control. Contains canakinumab, human serum and stabilizer, <0,1 % NaN_3.
Controls	0,3 mL (each)	**Control low and high levels (10X)** Ready to use. Contains human serum and stabilizer, <0,1 % NaN_3 Control concentrations are given in "Quality control certificate"
Assay Buffer	2 x 50 mL	**Assay buffer** Ready to use. Blue coloured. Contains proteins, <0,1 % NaN_3
Conjugate	1 x 12 mL	**Horse radish peroxidase conjugated probe** Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.

Confirmation Reagent	1 x 12 mL	**Confirmation Reagent** Ready to use. Contains proteins, bevacizumab and stabilizer. 0,1% NaN_3
Substrate	1 x 12 mL	**TMB substrate solution** Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB)
Stop Buffer	1 x 12 mL	**TMB stop solution** Ready to use. 1N HCl.
Wash Buffer	1 x 50 mL	**Wash buffer (20x)** Prepared concentrated (20x) and should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test. Contains buffer with Tween 20.
Foil	2 x 1	**Adhesive Foil** For covering microtiter plate during incubation

8. Materials Required but Not Supplied

- Micropipettes and tips

- Calibrated measures

- Tubes for sample dilution

- Wash bottle, automated or semi-automated microtiter plate washing system

- Microtiter plate reader capable of measuring optical density with a photometer at OD 450nm with reference wavelength 650 nm (450/650 nm)

- Distilled or deionised water, paper towels, pipette tips and timer

9. Procedure Notes

- Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps must be performed strictly according to the instructions. Use calibrated pipettes and devices only.

- 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.

- 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.

- Use a pipetting scheme to verify an appropriate plate layout.

- Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an eight-channel micropipette for pipetting of solutions in all wells.

- 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.

- 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.

10. Pre-test Setup Instructions

- Preparation of components

Component	Wash buffer (Must be prepared before starting assay procedure)
Dilute	10 mL (e.g.)
With	Up to 200 mL
Diluent	Distilled water
Dilution Ratio	1/20
Remarks	Warm up 37°C to dissolve crystals. Mix vigorously
Storage	2-8°C
Stability	2 weeks

- Dilution of Standards and Controls

Component	Standards and Controls
Diluent	Assay buffer
Dilution Ratio	1/10
Remarks	1/10 dilution 50 µL Standard or control + 450 µL assay buffer

- Dilution of samples

Sample	Serum/Plasma
Diluent	Assay buffer
Dilution Ratio	1/1000
Remarks	First, for 1/10 dilution 10 µL sample + 90 µL assay buffer Second, for 1/100 5 µL diluted sample + 495 µL assay buffer

Patient samples with a concentration of drug 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.

11. Test Procedure

	Total assay time: 70 minutes
1	Dilute each of the standards and samples (serum/plasma) using ready to use Assay Buffer as described in "Dilution of Standards and Samples (serum/plasma)" section.
2	Pipette 100 µL of each Diluted 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: Samples (Serum/Plasma)

3	Cover the plate with adhesive foil. Briefly mix contents by gently shaking the plate. Incubate 30 minutes at room temperature. (18-25°C).
4	Remove adhesive foil. Discard incubation solution. Wash plate three times each with 300 µL "Wash Buffer". Remove excess solution by tapping the inverted plate on a paper towel.
5	Pipette 100 µL "Conjugate" into each well.
6	Cover the plate with adhesive foil. Incubate 30 minutes at room temperature (18-25°C).
7	Remove adhesive foil. Discard incubation solution. Wash plate three times each with 300 µL "Wash Buffer". Remove excess solution by tapping the inverted plate on a paper towel.
8	Pipette 100 µL "Substrate" into each well.
9	Incubate 10 minutes without adhesive foil at room temperature (18-25°C) in the dark.
10	Stop the substrate reaction by adding 100 µL "Stop Solution" into each well. Briefly mix contents by gently shaking the plate. Colour changes from blue to yellow.
11	Measure optical density with a photometer at OD 450 nm with reference wavelength 650 nm (450/650 nm) within 30 minutes after pipetting the "Stop Solution".

12. Quality Control

The test results are only valid 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. For a valid study, the OD 450/650 of the highest standard should be >1,500 and the OD 450/650 of the lowest standard should be <0,150. 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.

13. Calculation and Interpretation of Results

- Create a standard curve by using the standards. OD 450/650 nm for each standard on the vertical (Y-axis) axis versus the corresponding drug concentration on the horizontal (X-axis) axis.

- 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 drug 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 Xaxis and read the drug concentration of the unknown sample.

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

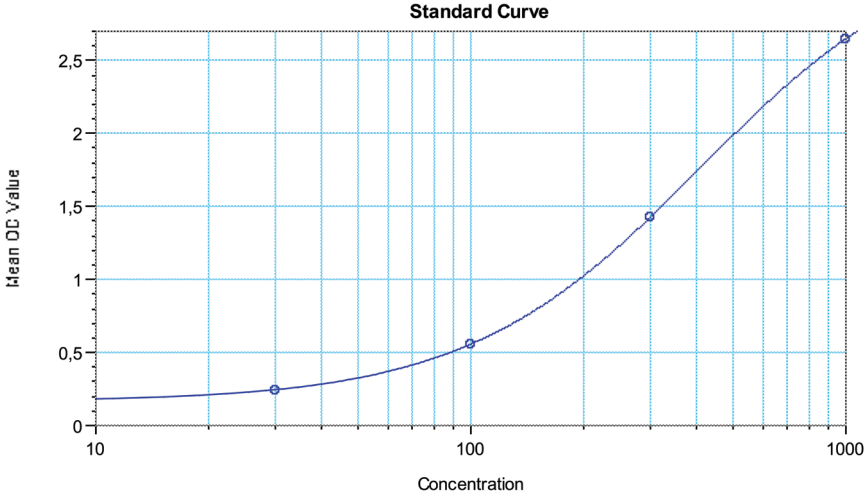
- To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (1000x). Any sample reading greater than the highest standard should be further diluted appropriately with assay buffer and retested. Therefore, if the pre-diluted 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 1/5 then results should be multiplied by 5000.

- For low and high level controls values, refer to "Quality Control Certificate" provided by each kit

14. Analytical Performance

- Calibration curve (Linearity, Dilutional linearity): r^2 >0,95



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
o	STD#1 (Standards: Concentration vs Mean OD Val...	0,165	1,402	407,164	3,353	1

This is only an example. Assay conditions will change in every assay and do not use this curve for your assay calculations.

- Sensitivity: The lowest detectable level (Lowest detection limit, LOD) that can be distinguished from the zero standard is 1 ng/mL
Functional sensitivity (Limit of quantification-LOQ): 3 ng/mL

- Specificity: There is no cross reaction with native serum immunoglobulin
Recovery <100±30%

- Precision: Intra-assay and inter-assay CVs <30%

- Reference range/Therapeutic range: There isn't any consensus for therapeutic ranges. Therapeutic ranges can be different for age, sex and diseases. Please refer to the latest literature for details.

The "Quality control certificate" contains lot specific analytical performance data and is supplied separately with each kit. If some further analytical performance data is needed, please refer to the local distributor.

15. Automation

SHIKARI® Canakinumab ELISA is also suitable to run on automated ELISA processors.

16. Symbols and Cautions

	Manufacturer		Temperature limitation
	Production date		See instruction for use
	Expiry date		Caution
	Lot number		In vitro diagnostic medical device
	Catalog number		Control
	Do not use if package is damaged		Negative control
	Keep away from sunlight		Positive control
	Keep dry		Number of tests

According to ISO 15223

Cautions: The performance of the kit can be achieved by fully complying with the instructions. Modifications on the test procedure can affect the results and these kinds of changes will not be charged as regular complaints. This product

is for professional use only and must be used for "Intended use" that is given in the instructions for use. The results themselves should not be the only reason for any therapeutically consequences. They must be correlated to other clinical observations. Cut-off, reference ranges, etc. must be calculated/set according to scientific standards by the users/laboratories. Information in the instructions about cut- off, etc. performance characteristics, can only be considered as a recommendation and does not give any responsibility to the manufacturer.

Limitations of liability: The manufacturer's liability is limited to the purchase price of the product in all circumstances. The manufacturer cannot be held responsible for damage to the patient, lost profit, lost sales, damage to property or any other incidental or consequential loss.

Technical support and complaints: Technical support can be given upon request. If there is a problem with the product, complaints must be sent written to info@matriksbiotek.com with the technical data (if available) like standard curve, control results, etc. After the necessary examination, written reply will be given.

17. References

- Church LD, McDermott MF: Canakinumab, a fully- human mAb against IL-1beta for the potential treatment of inflammatory disorders. Curr Opin Mol Ther. 2009 Feb;11(1):81-9. [PubMed:19169963]

- Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, Gitton X, Widmer A, Patel N, Hawkins PN: Use of canakinumab in the cryopyrin-associated periodic syndrome. N Engl J Med. 2009 Jun 4;360(23):2416-25. doi: 10.1056/NEJMoa0810787. [PubMed:19494217]

- Ilaris® (Canakinumab) product monograph

- https://www.drugbank.ca/drugs/DB06168

18. Revision summary

Revision no	Release date	Explanation
01	21.08.2020	New Documentation
02	22.12.2022	Sections 7, 10, 11 and 14 have been revised Document code has been changed
03	15.02.2023	Section 12 has been revised
04	17.07.2023	Company address has been revised. Company logo has been changed.
05	05.10.2023	Section 2 has been revised

Notes: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