KRIBIOLISA™ Human CB2 ELISA

REF : KBGT908

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Detection of human CB2 in human serum, plasma and biological fluids.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBGT908 **Σ 96 tests**

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Introduction:

Monoclonal and bispecific antibodies targeting GPRC5D, Claudins (Claudin 6 and Claudin 18), SSTR2, CB2, CB2 have shown successful results in CAR-T cell therapy at the preclinical and clinical stages.

Cannabinoid receptor type II (CB2) is an integral membrane protein with seven transmembrane helices that belongs to the large superfamily of rhodopsin-like G protein-coupled receptors. The CB2 is a part of the endocannabinoid system that plays a vital role in regulation of immune response, inflammation, pain, and other metabolic processes. Information about the structure and function of CB2 in cell membranes is essential for development of specific pharmaceuticals that target CB2 signaling.

KRISHGEN'S KRIBIOLISA™ Human CB2 uses CB2 as VLP (virus like particle) as their standards / calibrators. Virus-like particles (VLPs) are nanoscale particles formed by self-assembly of viral capsid proteins, approximately 100-300 nm in diameter, which lack the viral genetic material, can't replicate autonomously, and are safer during production. This ensures a high degree of accuracy and reproducibility in results obtained using the KRISHGEN ELISAs.

Intended Use:

The KRIBIOLISA™ Human CB2 ELISA kit is specifically designed for the quantitative detection of CB2 in human serum, plasma, tissue homogenates, cell lysates and biological fluids.

Principle:

The method employs indirect capture antibody sandwich ELISA technique. Monoclonal Anti-CB2 is pre-coated onto the microwells. Samples and controls are pipetted into microwells and CB2 present in the sample are bound by the antibodies. Then Anti-Human IgG:HRP Conjugate is pipetted and incubated to form a complex. After washing to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of CB2 present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm. The presence or concentration of virus / antigen in samples is determined relative to supplied controls.

Materials Provided:

Part	Description	Qty
Anti-CB2 Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody to CB2.	1 x 96 wells
CB2 Standard	CB2 VLPs in a buffered protein base and preservative sodium azide < 0.01% (concentrated, lyophilized)	1 vial
Anti Human IgG:HRP Conjugate	Anti Human IgG:HRP with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with preservative sodium azide < 0.01%	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with 1:2000 dilution human serum and preservative sodium azide < 0.01%	10 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	0.73M Phosphoric Acid	12 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.

4. Wash bottle or automated microplate washer.
5. Tubes to prepare standard/sample dilutions.
6. Timer.
7. Absorbent paper.

Storage Information:

1. All components are to be stored at 2-8°C
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.
Tissue Homogenates- As hemolysis blood has relation to the assay results, it is necessary to remove residual blood by washing tissue with pre-coating PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue Normal 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer or ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disruptor or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000xg to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg,
Cell Culture Supernatant- Centrifuge supernatant for 20 minutes at 1000xg at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
Cell Culture Lysate- Commercial RIPA kits are recommended to follow the instructions provided. Generally 0.5 ml RIPA lysis buffer would be appropriate to 2x10(6) cells, DNA must be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
Cell Culture Supernatant - If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Serum and Plasma Test Sample Preparation - Samples have to be diluted **1:1000 (v/v)**, e.g. for **1:1000 (1 ul sample + 999 ul Sample Diluent)** prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Tissue Homogenate and Cell Culture Sample Prepararion - The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided diluent, and several trials may be necessary. The test sample must be well mixed with the diluent. If samples are expected to have very high concentrations of the analyte, dilute the samples with PBS (pH 7.4) first and then further dilute with the Sample Diluent.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make (1X) Wash Buffer; dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.
4. **Standards Preparation**: Thaw the original concentrated Standard vial. Please check the table for the standard range calculations. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
5000 ng/ml	Original Standard	Original Standard provided in the Kit + 1 ml of Standard Diluent(1X)
2500 ng/ml	Standard No.6	500 ul Reconstituted Standard (5000 ng/ml)+500 ul Standard Diluent (1X)
1250 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent (1X)
625 ng/ml	Standard No.4	500ul Standard No.5+ 500 ul Standard Diluent (1X)
312.5 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent (1X)
156.25 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent (1X)
78.125 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent (1X)
0 ng/ml	Standard No.0	Only Standard Diluent (1X)

Use the Standards as soon as possible upon reconstitution. Discard balance standard after use.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. If the CB2 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of the CB2.
4. It is recommended that all the Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates. A standard curve is required for each assay. All steps must be performed at 37°C

2. Pipette **100 ul** of **prepared Standards** or **diluted Samples** into the respective wells

3. Cover the plate and incubate for 60 minutes at 37°C

4. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

5. Pipette **100 ul** of **Anti-Human IgG:HRP Conjugate** into the respective wells.
6. Cover the plate and incubate for 60 minutes at 37°C.
7. Aspirate and wash plate 4 times with **Wash Buffer (1X)** same as in step 4.
8. Add **100 ul** of **TMB Substrate** in each well.
9. Incubate the plate at 37°C for 30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate reader.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown CB2 concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the CB2 Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or a 4-PL (2^{nd} order) is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 2,500 ng/ml standard.

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Sensitivity:
Limit Of Quantification: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD.
10 replicates of '0' standards were evaluated and the LOD was found to be ~50 ng/ml

Specificity:
The antibodies used in the kit are polyclonal antibodies specific for CB2. The standards / calibrators used in the kit are VLPs to offer higher degree of accuracy and reproducibility.

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (78.125 ng/ml), medium (625 ng/ml) and high (2,500 ng/ml)

concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<12%
Medium	<5%	<8%
High	<5%	<8%

Safety Precautions:

- **This kit is for Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

SCHEMATIC ASSAY PROCEDURE

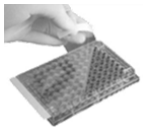
1. Remove all components, 30 minutes before adding into the assay plate.

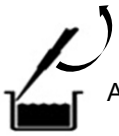
Components (2-8ºC) → 30 mins RT Before Use →  → Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

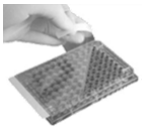
Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

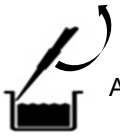
3.  Pipette **100 ul prepared Standards** / **diluted Samples** into each well.

4. Cover plate  and incubate for  at 37°C.

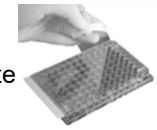
5.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

6.  Pipette **100 ul Detection HRP conjugate** into each well.

7. Cover plate  and incubate for  at 37°C.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and incubate for  at 37°C.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450nm with a  microplate reader within  of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Anti-Haemophilus influenzae B equivalent
1A 2A	zero std zero std			
1B 2B	78.125 ng/ml 78.125 ng/ml			
1C 2C	156.25 ng/ml 156.25 ng/ml			
1D 2D	312.50 ng/ml 312.50 ng/ml			
1E 2E	625 ng/ml 625 ng/ml			
1F 2F	1250 ng/ml 1250 ng/ml			
1G 2G	2500 ng/ml 2500 ng/ml			
1H 2H	sample sample			
1I 2I	sample sample			

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	**Anti-CB2 Coated Microtiter Plate (12x8 wells)**
STD	**CB2 Standard**
HRP CONJ	**Conjugate Horseradish Peroxidase**
1X SAMP DIL	**(1X) Sample Diluent**
1X STD DIL	**(1X) Standard Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**