






KRIBIOLISA™ Carbamazepine (TEGRETOL) ELISA

REF: KBBA22

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Carbamazepine in human serum and plasma

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

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

REF KBBA22

 **96 tests**



KRISHGEN BioSystems

For US / Europe: toll free +1(888)-970-0827 tel: +1(562)-568-5005
For Asia / India: tel: +91(22)-49198700
Email: sales@krishgen.com

Introduction:

Carbamazepine, sold under the trade name Tegretol, is an anticonvulsant medication used primarily in the treatment of epilepsy and neuropathic pain. It is used as an adjunctive treatment in schizophrenia along with other medications and as a second-line agent in bipolar disorder. It is on the World Health Organization's List of Essential Medicines. In 2018, it was the 204th most commonly prescribed medication in the United States, with more than 2 million prescriptions.

Intended Use:

The KRIBIOLISA™ Carbamazepine (TEGRETOL) ELISA is used as an analytical tool for quantitative determination of Carbamazepine in human serum and plasma.

Principle:

The method employs the competitive enzyme immunoassay technique. In the first step, samples and standards are pipetted into microwells. If Carbamazepine is present in the samples or standards, it will form complex with specific antibodies which is pre-coated onto microwells. In the second step, Carbamazepine:HRP conjugate is pipetted and incubated. Free HRP conjugate will be removed by a washing step. In the third step, TMB substrate is added to microwells and color develops inversely proportionally to the amount of Carbamazepine present in the samples or standards. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Anti-Carbamazepine monoclonal Antibody Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Carbamazepine monoclonal Antibody.	1 x 96 wells
Recombinant Carbamazepine Standard	Recombinant Carbamazepine Standard (concentrated. 1 mg/ml)	1 vial
(1X) Standard Diluent	Buffered protein base with 1:1000 human serum and with preservative thiomersol <0.01%	10 ml
Carbamazepine:HRP Conjugate	Carbamazepine conjugated to horseradish peroxidase with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with preservative thiomersol <0.01%	2 x 50 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	2N Sulfuric 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. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components 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.
3. After reconstitution of standards, it has to be used immediately and cannot be stored.

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 serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Preparation before Use:

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

Test Sample preparation -Samples have to be diluted 1 in 1000 (v/v), e.g. 1 ul sample in 999 ul of (1X) 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.

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. High Dose Hook Effect may be observed in samples with very high concentrations of Carbamazepine. High Dose Hook Effect is due to excess of antibody for very high concentrations of Carbamazepine present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Carbamazepine concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of Carbamazepine.
4. It is recommended that all 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. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

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. The Main standard Concentration is as per accompanying Reagent Sheet available in the kit. Prepare standards as per instructions and use as indicated.

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. High Dose Hook Effect may be observed in samples with very high concentrations of Carbamazepine. High Dose Hook Effect is due to excess of antibody for very high concentrations of Carbamazepine present in the sample.
3. Avoid assay of Samples containing sodium azide (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of Carbamazepine.
4. It is recommended that all 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. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Add 100 ul of **prepared Standards and samples** into their respective well.
2. Incubate at 37°C for 60 minutes.
3. Aspirate and wash plate 5 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.
4. Add 100 ul of **Carbamazepine:HRP Conjugate** into each well.
5. Incubate at 37°C for 30 minutes.
6. Aspirate and wash plate 5 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.
7. Dispense 100 ul per well of **TMB Substrate**. Cover the plate.
8. Incubate at Room Temperature under dark for 15-30 minutes.
8. Add 100 ul of **Stop Solution** to each microwell.
9. Measure the optical density of the wells on a plate reader at 450 nm within 10 minutes.

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Using semi-log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown Carbamazepine concentrations, find the unknowns 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 Carbamazepine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software; 4-PL or cubic spline or polynomial regression (2nd order) may be preferred.

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.

Specificity:

The standard / calibrator used in the kit are calibrated against commercially sourced Tegretol®

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.



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 product; 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 product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

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

Krishgen Biosystems. 2022

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS®, GENLISA®, DHARMAPLEX™, GENBULK™, GENLISA™, KRISHZYME®, KRISHGEN®, KRIBIOLISA®, KRISHPLEX®, TITANIUM®, QUALICHEK® are registered trademarks of KRISHGEN BIOSYSTEMS. ©KRISHGEN BIOSYSTEMS. ALL RIGHTS RESERVED.

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |