KRIBIOLISA™ Glatiramer Acetate (COPAXONE™) ELISA

REF : KOD1024

Ver.5.2

RUO

Enzyme Immunoassay for the Quantitative Determination of Glatiramer Acetate in cell culture supernatant and pharmaceutical preparations

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

For Research Use Only. 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.

KOD1024 **96 tests** **REF** Σ

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:

Glatiramer Acetate (Copaxone™) is a mixture of random-sized peptides that are composed of the four amino acids found in myelin basic protein, namely glutamic acid, lysine, alanine, and tyrosine. Myelin basic protein is the antigen in the myelin sheaths of the neurons that stimulates an autoimmune reaction in people with multiple sclerosis (MS), so the peptide may work as a decoy for the attacking immune cells.

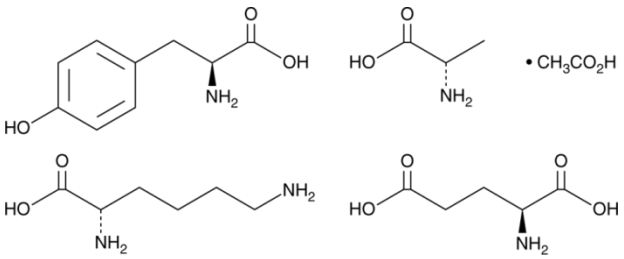
Trade Names:
Copaxone, Glatect, Glatopa

Generic Name:
Glatiramer

PubChem CID:
Not available

DrugBank Accession Number:
DB05259

Structure Depiction:

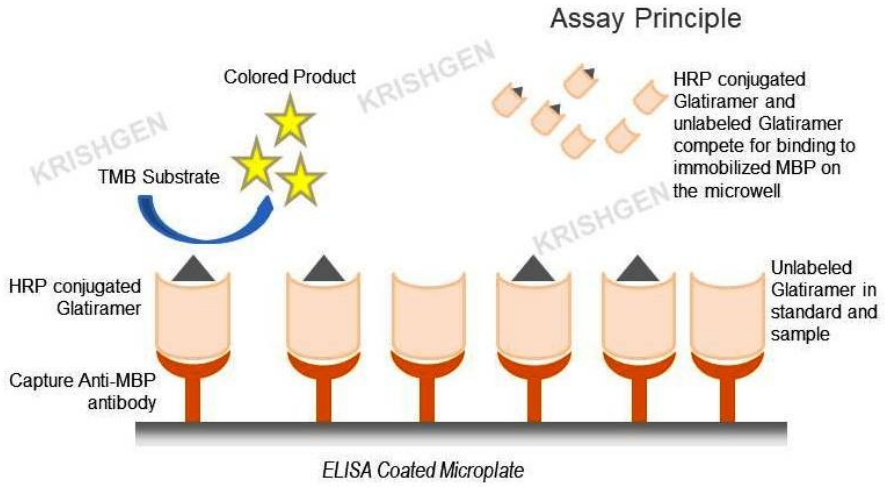


Intended Use:

The KRIBIOLISA™ Glatiramer Acetate (COPAXONE™) ELISA kit is used for the quantitative estimation of Glatiramer Acetate in cell culture supernatant and pharmaceutical preparations.

Principle:

The Glatiramer Acetate ELISA is a competitive immunoassay for the determination of Glatiramer Acetate (GA). It is known that Glatiramer binds strongly to MBP-specific antigen. Hence using this principle the assay has been developed. A varying concentration of unlabeled standard or sample and constant concentration of GA:HRP conjugate will bind in sequence to the antibodies coated on the microplate. Upon washing, unbound GA:HRP Conjugate will be removed. Bound GA:HRP complex will produce a soluble blue colored product after the addition of TMB Substrate. The enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is inversely proportional to the amount of bound Glatiramer Acetate present in the standards or samples.



Materials Provided:

Part	Description	Qty
Anti-MBP polyclonal antibody Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-MBP polyclonal antibody Coated Microtiter Plate	1 x 96 wells
Glatiramer Acetate standard	Glatiramer Acetate standard (lyophilized, concentration - 10,000 ng/ml)	2 vials

Part	Description	Qty
Glatiramer Acetate (GA) HRP (conc)	Glatiramer Acetate (GA) HRP (concentrated, liquid)	10 ul
(1X) Conjugate Diluent	Buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
Assay Diluent	Buffered protein base with preservative thiomersol < 0.01%	50 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane with 1:5000 dilution human serum	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. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

For 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.

For Pharmaceutical Preparations - If necessary, centrifuge to remove debris / un-dissolved matter prior to analysis. Dissolve in Assay Diluent to the desired concentration to avoid matrix or dilutional linearity effect. Samples may be stored at recommended temperature.

Preparation Before Use:

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

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**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent to obtain 10,000 ng/ml. Keep the vial for 15 mins with gentle agitation and then run the assay procedure. Use the Standard Diluent as the zero standard. Below table shows the calculation for the standard range.

Standard Concentration (ng/ml)	Standard No.	Dilution Particulars
10,000 ng/ml	Lyophilized standard	Lyophilized Standard + 1ml of Standard Diluent
1000 ng/ml	Standard No.8	100 ul Reconstituted Standard + 900 ul Standard Diluent
500 ng/ml	Standard No.7	500 ul Standard No.8 + 500 ul Standard Diluent
250 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
125 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
62.5 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
31.25 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
15.6 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
7.8 ng/ml	Standard No.1.	500 ul Standard No.2 + 500 ul Standard Diluent
0 ng/ml	Standard No.0	500 ul Standard Diluent

Mix each tube thoroughly before the next transfer. Use the standards for experiment within one hour of preparation of standard. Discard standard after use.

5. **Working Glatiramer Acetate:HRP Conjugate -** Prepare the working stock as per table below.

Procedure Step No		GA:HRP Conjugate (conc)	Diluted Stock from Step 1	Conjugate Diluent	Total Volume	No of Wells to be Used
1	Pipette GA:HRP Conjugate (conc)	1 ul		999 ul	1000 ul	
2	Mix well by pipetting					
3	Add from Step 2 diluted solution to prepare Working GA:HRP Conjugate		1 part	15 parts	16 parts	
	for example from step 2, pipette		60 ul	900 ul	960 ul	8 wells
			120 ul	1800 ul	1920 ul	16 wells
			320 ul	4800 ul	5120 ul	48 wells
			700 ul	10500 ul	11200 ul	96 wells

Note: the diluted Conjugate is extremely unstable from both step 1 and step 2. Please do not aliquot to store for later use. Use the prepared diluted Conjugate immediately for your assay.

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. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity of the conjugate resulting in under-estimation of the antibodies.
3. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. 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.
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.
8. Making serial dilution in the wells directly is not permitted.
9. Prepare the Standard within 15 minutes prior to running the assay.
10. Please carefully dilute Standards according to the instruction, and avoid foaming. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettes are calibrated.
11. If crystals have formed in the Wash Solution (20X) concentrate, warm to room temperature and mix gently until the crystals are completely dissolved.
12. Contaminated water or container for reagent preparation will influence the detection results.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at Room Temperature.

2. Pipette out **100 ul of Standards and samples** to the respective wells.

3. Add **100 ul Working Glatiramer Acetate:HRP Conjugate** to each well.

4. Cover the plate and incubate for 90 mins at Room Temperature.

5. 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.

6. Add **100 ul** of **TMB Substrate** in each well.

7. Incubate the plate at RT 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.

8. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

9. 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. Calculate percent conjugate bound (%B) for each standard and sample relative to the maximum binding (B0, Zero standard) wells as follows:

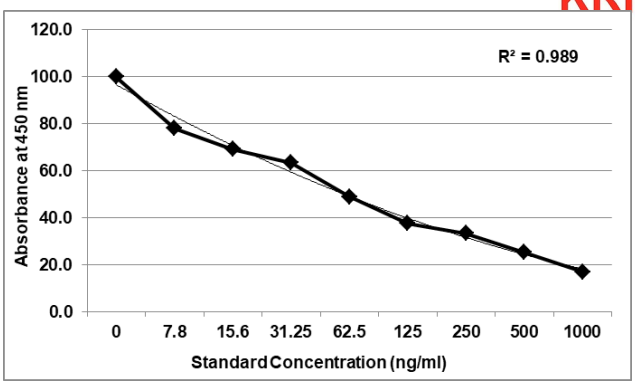
%B/B0 = Mean Absorbance (Standard / Sample) / Mean Absorbance (Zero ‘0’ Standard) x 100

Using a semi-log graph paper, plot the B/Bo (%) for each standard point (y axis) versus the concentration (x axis). Draw a best-fit line (logit-log or 4-PL) through the points. To determine the concentration of your samples, find the B/Bo (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of your Glatiramer Acetate in the sample. If samples were diluted, multiply by the appropriate dilution factor.

Typical Data

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration	%B/B0
0	2.507	--	--	100.0
7.8	1.958	7.9	101.2	78.1
15.6	1.734	16.8	107.5	69.2
31.25	1.591	25.5	81.6	63.5
62.5	1.229	68.2	109.1	49.0
125	0.943	150.1	120.1	37.6
250	0.834	213.0	85.2	33.3
500	0.634	434.3	86.9	25.3
1000	0.427	1192.7	119.3	17.0

Typical Graph



abs = absorbance at 450nm

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:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Detection Range:
7.8 ng/ml - 1000 ng/ml.

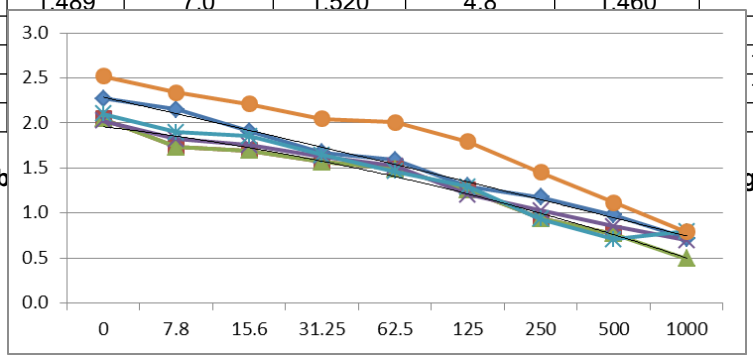
Sensitivity:
The minimum detectable dose of Glatiramer Acetate is <50 ng/ml.

Precision:
Intra-Assay Precision: 3 samples with low, middle and high level human Glatiramer Acetate were tested 20 times on one plate, respectively.
Inter-Assay Precision: 3 samples with low, middle and high level human Glatiramer Acetate were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/mean X 100
Intra-Assay: CV<15%
Inter-Assay: CV<18%

Stability Profile of GA:HRP Conjugate (conc):
2 kits were run with the GA:HRP Conjugate (conc) kept at 37^C to run an accelerated stability study to see the stability of the GA:HRP Conjugate (conc). The GA:HRP Conjugate (conc) was freshly diluted as per IFU prior to each run. All components used were common through and kept at 2-8^C as per prescribed storage instructions. The GA:HRP Conjugate (conc) was stable till the expiry date.

Standards	Day 0	Day 1	Std Dev %	Day 4	Std Dev %	Day 7	Std Dev %	Day 11	Std Dev %
		26 days		104 days		182 days		286 days	
0	2.270	2.046	15.8	2.025	17.3	2.101	12.0	2.517	17.5
7.8	2.151	1.734	29.5	1.821	23.3	1.896	18.0	2.339	13.3
15.6	1.910	1.693	15.4	1.752	11.2	1.850	4.2	2.211	21.3
31.25	1.674	1.568	7.5	1.625	3.4	1.644	2.1	2.048	26.5
62.5	1.588	1.489	7.0	1.520	4.8	1.460	9.1	2.007	29.6
125	1.299						0.6	1.795	35.1
250	1.173						17.0	1.452	19.7
500	0.979						19.1	1.116	9.7
1000	0.719						5.4	0.789	4.9



Stab **gate (conc)**

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.

Reference:

Antibodies to glatiramer acetate do not interfere with its biological functions and therapeutic efficacy… Teitelbaum D, Brenner T, Abramsky O, Aharoni R… Mult Scler…2003…SAGE

Blocking effects of serum reactive antibodies induced by glatiramer acetate treatment in multiple sclerosis… Hassan H. Salama Jian Hong Ying C. Q. Zang Azza El-Mongui Jingwu Zhang… Brain…2003…Oxward academic

The Effect of Glatiramer Acetate Therapy on Functional Properties of B Cells From Patients With Relapsing-Remitting Multiple Sclerosis…Sara J. Ireland, Alyssa A. Guzman, Dina E. O'Brien…JAMA Neurol..2014

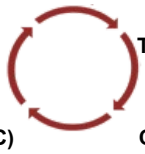
SCHEMATIC ASSAY PROCEDURE

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

Components (2-8ºC) → 30 mins RT Before Use → 

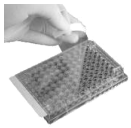
Thaw at Room Temperature (18-24ºC) → USE NOW

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

3.  Pipette **100 ul prepared Standard** and **Sample** into respective well.

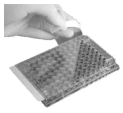
4.  Pipette **100 ul Working GA:HRP conjugate** into the respective wells.

5. Cover plate  and incubate for 

at Room Temperature.

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

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

8. Cover plate  and incubate for 

at Room Temperature.

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

10. 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 Glatiramer Acetate
1A 2A	zero standard zero standard			
1B 2B	7.8 ng/ml 7.8 ng/ml			
1C 2C	15.6 ng/ml 15.6 ng/ml			
1D 2D	31.25 ng/ml 31.25 ng/ml			
1E 2E	62.5 ng/ml 62.5 ng/ml			
1F 2F	125 ng/ml 125 ng/ml			
1G 2G	250 ng/ml 250 ng/ml			
1H 2H	500 ng/ml 500 ng/ml			
3A 4A	1000 ng/ml 1000 ng/ml			
3B 4B	Sample			
3C 4C	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 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. 2023

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

Copaxone is the registered trade mark of Teva Pharmaceuticals.

SYMBOLS KEY

MTP	Anti-MBP polyclonal antibody Coated Microtiter Plate (12x8 wells)
STD	Standard
HRP CONJ	Conjugate Horseradish Peroxidase (conc)
DETN DIL	Detection diluent
ASY DIL	Assay 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