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KRIBIOLISA™ CRM197 ELISA

REF : KBVH097

Ver 2.0

RUO

Enzyme Immunoassay for the Quantitative Determination of CRM197 in cell culture supernatant, vaccines and biological 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.

REF KBVH097

96 tests

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email: sales@krishgen.com | http://www.krishgen.com

Introduction:

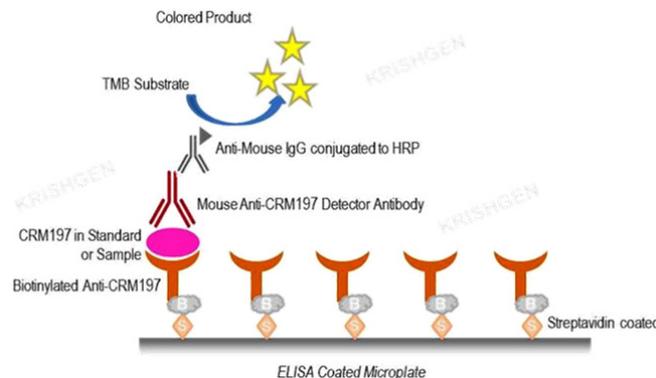
The KRIBIOLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

CRM197 is a genetically detoxified form of diphtheria toxin. A single mutation at position 52, substituting glutamic acid for glycine, causes the ADP-ribosyltransferase activity of the native toxin to be lost. CRM197 is widely used as a carrier protein for conjugate vaccines. A potential advantage of CRM197 over toxoided proteins is that, because it is genetically detoxified, it retains its full complement of lysine amines for conjugation. The KRIBIOLISA™ Diphtheria Toxin CRM197 ELISA kit is used as an analytical tool for quantitative determination of Diphtheria Toxin CRM197 in culture supernatant, vaccines and other biological preparations.

Principle:

The method employs sandwich ELISA technique. Plates are coated with Streptavidin. Biotinylated Anti-CRM197 antibody is added to the plate and incubated. After incubation wells are washed with wash buffer and standard and sample are added to the wells. After incubation wells are washed with wash buffer and Anti-CRM197 antibody is added. After incubation, again wells are washed and Anti-Mouse IgG HRP conjugate is added. Complex is formed. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Diphtheria Toxin CRM197 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

Part	Description	Qty
Streptavidin Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Streptavidin.	1 x 96 wells
CRM197 Standard	Diphtheria CRM197 Standard in a buffered protein base with preservative sodium azide - lyophilized (200 ug/ml)	2 vials
Biotin labeled Anti-CRM197 Antibody	Biotin labeled Anti-CRM197 antibody with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
Mouse Anti-CRM197 Detector Antibody	Mouse Anti-CRM197 detector antibody with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
Anti-Mouse IgG:HRP conjugate	Anti-Mouse IgG:HRP conjugate with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Standard Diluent	Buffered protein base with 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	2N Sulfuric Acid	12 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2°C to 8°C for stability.
2. All the reagents and wash solutions should be used within 6 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25°C). 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:**

Cell Culture Supernatant and Biological Preparations including Vaccines: Centrifuge supernatant for 20 minutes at 1000×g at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.

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 CRM197. High Dose Hook Effect is due to excess of antibody for very high concentrations of CRM197 present in the sample.
3. CRM197 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of CRM197.
5. It is recommended that all Standards and Samples be assayed in duplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromise of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. 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. To make **Wash Buffer (1X)**; dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**.
4. **Standards Preparation:** Reconstitute the concentrated Standard lyophilized vial with 250 ul of Standard Diluent to obtain a concentration of 800ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Prepare **Standards** as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
800 ug/ml	Lyophilized Standard	Lyophilized Standard in the Kit + 250 ul of Standard Diluent (1X)
800 ug/ml	Standard No.8	Reconstituted Standard
400 ug/ml	Standard No.7	125 ul Reconstituted Standard (800 ug/ml) + 125 ul Standard Diluent (1X)
200 ug/ml	Standard No.6	125 ul Standard No.7 + 125 ul Standard Diluent (1X)
100 ug/ml	Standard No.5	125 ul Standard No.6 + 125 ul Standard Diluent (1X)
50 ug/ml	Standard No.4	125 ul Standard No.5 + 125 ul Standard Diluent (1X)
25 ug/ml	Standard No.3	125 ul Standard No.4 + 125 ul Standard Diluent (1X)
12.5 ug/ml	Standard No.2	125 ul Standard No.3 + 125 ul Standard Diluent (1X)
6.25 ug/ml	Standard No.1	125 ul Standard No.2 + 125 ul Standard Diluent (1X)
0 ug/ml	Standard No.0	Only Standard Diluent

Use the Standards immediately upon reconstitution. Discard balance standard after use. Do not store them for further experiments.

Assay Procedure:

1. A standard curve is required for each assay. All steps must be performed at 37°C
2. Add **100 ul Biotin labeled Anti-CRM antibody solution** to the respective well. Mix well.
3. Cover the plate with a sealer and incubate for **60 minutes at 37°C**.
4. Aspirate and wash plate 4 times with diluted 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. Add **100 ul prepared Standard or Samples** to the respective wells.
6. Incubate for **30 minutes at 37°C**.
7. Aspirate and wash plate 4 times with diluted **(1X) Wash Buffer** as described in step 4.
8. Pipette **100 ul Mouse Anti-CRM Detector Antibody** to all the wells. Mix well.
9. Cover the plate with a sealer and incubate for **60 minutes at 37°C**.
10. Aspirate and wash plate 4 times with diluted **(1X) Wash Buffer** as described in step 4.
11. Pipette **100 ul Rabbit Anti-Mouse IgG:HRP conjugate solution** to all the wells.
12. Incubate for **30 minutes at 37°C**.
13. Aspirate and wash plate 4 times with diluted **(1X) Wash Buffer** as described in step 4.
14. Pipette **100 ul TMB Substrate** in all the wells.
15. Incubate the plate at 37°C for 30 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
16. Pipette **100 ul of Stop Solution** in all wells. The wells should turn from blue to yellow in color.
17. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) 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 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 Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4PL (2nd order) is best recommended for automated results.

Note:

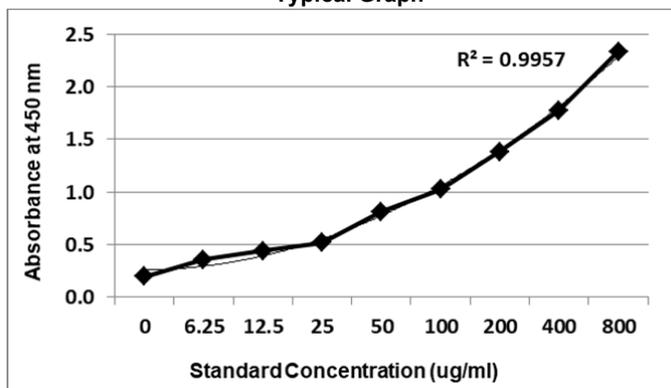
It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 800 ug/ml standard.

Typical Data

Standard Concentration (ug/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.195	0.1	--
6.25	0.356	6.3	100.8
12.5	0.440	12.3	98.3
25	0.517	20.1	80.4
50	0.810	58.5	116.9
100	1.022	100.4	100.4
200	1.384	205.1	102.6
400	1.776	381.4	95.4
800	2.332	812.7	101.6

Typical Graph



Abs = absorbance at 450nm

Assay Range:

6.25 ug/ml - 800 ug/ml

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 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 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.

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KEY SYMBOLS

MTP	Streptavidin Coated Microtiter Plate (8 x 12 wells)
STD	Diphtheria CRM Standard
HRP CONJ	Anti-Mouse IgG:HRP conjugate
BIO ANTB	Biotin labeled Anti-CRM antibody
DET ANTB	Mouse Anti-CRM Detector antibody
STD DIL	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

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