KRIBIOLISA® Vaccinia Capping Enzyme Residue Detection ELISA

REF : KBBA60

Ver 1.0

RUO

ELISA for Quantitative Determination of Vaccinia Capping Enzyme Residue Detection in Cell Culture and Biological Samples

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 KBBA60 Σ 96 tests

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Introduction:

In eukaryotes, mRNA is modified after transcription to form a special structure at the 5' end, i.e., the cap structure, which plays a more important role in the stabilization, transport and translation process of mRNA. Cowpox virus capsid enzyme is an effective enzyme that catalyzes the formation of the cap structure, which consists of two subunits, D1 and D12, and combines RNA triphosphate esterase activity, guanylate acyltransferase activity and guanine methyltransferase activity, which can connect the 7-methylguanine cap structure (m7Gppp) to the 5' end of RNA (m7Gppp5'N). Cowpox Virus Capping Enzyme, in the presence of appropriate concentrations of capping buffer, guanosine triphosphate (GTP), and S-adenosine methionine (SAM), is capable of capping RNAs in less than an hour and ensures proper orientation.

Intended Use:

The KRIBIOLISA® Vaccinia Capping Enzyme Residue Detection ELISA kit developed for quantitative determination of Vaccinia Capping Enzyme Residue Detection in Cell culture and Biological samples.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Vaccinia Capping Enzyme present in the sample are bound by the antibodies. Biotin labeled antibody is added and followed by Streptavidin-HRP is pipetted and incubated to form a complex. 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 Vaccinia Capping Enzyme in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Kit Contents:

1. Vaccinia Capping Enzyme Antibody Microtitre coated well plate (12 x 8 wells) - 1 no
2. Vaccinia Capping Enzyme Standard (concentrated, lyophilized, 32 ng/ml) – 2 vials
3. Biotinylated Vaccinia Capping Enzyme Antibody (concentrated) - 120 ul
4. Streptavidin:HRP Conjugate (concentrated) - 40 ul
5. Standard Diluent - 45 ml
6. Assay Diluent - 30 ml
7. Wash Buffer (20X) - 50 ml
8. TMB Substrate - 15 ml
9. Stop Solution - 10 ml
10. Instruction manual

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. Graduated cylinder, Beaker, Centrifuge tube of different volumes.
4. Deionized (DI) water.
5. Wash bottle or automated microplate washer.
6. Graph paper or software for data analysis.
7. Tubes to prepare standard/sample dilutions.
8. Timer.
9. Absorbent paper.

Storage Information:

1. Store the Standard and Detection Antibody at -20°C upon receipt. Rest all kit components shall 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 serum and/or plasma in accordance with NCCLS regulations.

Sample Preparation:

The test sample should be diluted at least 1:2 in Standard Diluent. At this dilution Standard Diluent interference in assay will be negligible.

Reagent Preparation:

1. Bring all reagents and samples to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.
2. To make **Wash Buffer (1X) 500 ml;** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**.
3. **Detection Antibody** – Centrifuge at 10,000 x g for 20 seconds. Dilute to work concentration at 1:100 Assay Diluent before use.
4. **Streptavidin-HRP -** Centrifuge at 10,000 rpm for 20 sec before use, and then dilute the detection antibody by 1:500 with Assay Diluent to make a working solution.
5. **Standards preparation:** Reconstitute the Vaccinia Capping Enzyme Standard with 1 ml of Standard Diluent to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (**Do not turn the vial upside down**). Mix the standard to ensure complete reconstitution prior to making dilutions.

Standard Concentration	Standard Vial	Dilution Particulars
32 ng/ml	Original Standard	Reconstitute with 1.0 ml Standard Diluent
8 ng/ml	Standard No.8	150 ul Original Standard + 450 ul Standard Diluent
4 ng/ml	Standard No.7	300 ul Standard No.8 + 300 ul Standard Diluent
2 ng/ml	Standard No.6	300 ul Standard No.7 + 300 ul Standard Diluent
1 ng/ml	Standard No.5	300 ul Standard No.6 + 300 ul Standard Diluent
0.5 ng/ml	Standard No.4	300 ul Standard No.5 + 300 ul Standard Diluent
0.25 ng/ml	Standard No.3	300 ul Standard No.4 + 300 ul Standard Diluent
0.125 ng/ml	Standard No.2	300 ul Standard No.3 + 300 ul Standard Diluent
0 ng/ml	Standard No.1	300 ul Standard Diluent only

Assay procedures

Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack and reseal.
3. Wash each well three times with Wash Buffer (300 ul/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **C**omplete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add **100 ul** of each serially diluted standard or test sample per well. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate at 37°C for 60 minutes.
5. Wash each well five times with Wash Buffer (300 ul/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 ul of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 60 minutes at 37°C.
7. Repeat the aspiration/wash as in **Step 5.**
8. Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells. Mix well.
9. Cover the plate with a sealer and incubate for 40 minutes at 37°C.
10. Repeat the aspiration/wash as in **Step 5.**
11. Add **100 ul of TMB** solution to each well, seal plate and incubate for 15 minutes at 37°C. Protect from light.
12. Add Stop reaction by adding **50 ul of Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
13. Determine the optical density of each well within 20 minutes, using a microplate reader set to 450 nm and 630 nm, 450 nm as the detection wavelength and 630 nm as the reference wavelength.

Calculation of Results:

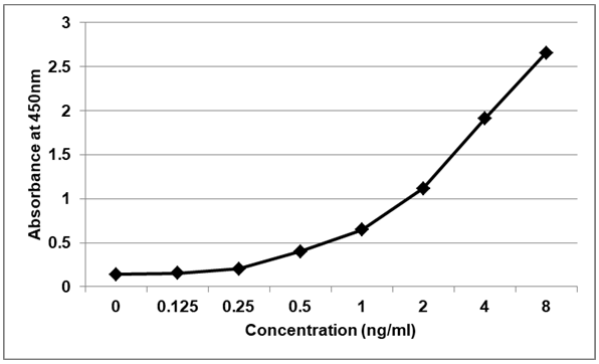
Determine the mean absorbance for each set of duplicate standards and samples. Plot the standard curve on graph paper, with concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown concentrations, find the unknowns 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 concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software may be preferred.

Typical Data

Standard Conc. (ng/ml)	OD		Mean OD	Calibration value
8	2.455	2.374	2.455	2.456
4	1.517	1.457	1.517	1.513
2	0.886	0.828	0.886	0.887
1	0.505	0.492	0.505	0.515
0.5	0.310	0.286	0.310	0.307
0.25	0.205	0.190	0.205	0.195
0.125	0.135	0.128	0.135	0.135
0	0.066	0.065	0.066	-

Typical Graph



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.

Sensitivity:
Limit of Quantification: It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 0.065 ng/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of Vaccinia Capping Enzyme. No significant cross-reactivity or interference between Vaccinia Capping Enzyme and analogues was observed.

Assay Range:
0.125 ng/ml – 8 ng/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.
- 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

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SYMBOLS KEY

MTP	Microtitre coated well plate (96 wells)
STD	Standard
BIOTIN AB	Biotinylated Antibody
HRP CONJ	Conjugate Horseradish Peroxidase
STD DIL	Standard Diluent
ASS DIL	Assay 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