Version 18-09/25

User's Manual

Vaccinia Capping Enzyme ELISA Kit

REF **DEIA-BZ003**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For the quantitative measurement of Vaccinia Capping Enzyme in biological products. It is applicable to theoptimization of purification process of biological products, impurity control of intermediate process and release testing of final products.

General Description

The cap structure is a modification at the 5' end of eukaryotic mRNA formed after transcription. Vaccinia capping enzyme is an effective catalyst in forming this cap structure. Therefore, for biological products utilizing vaccinia capping enzyme, residual levels must be quantitatively assessed.

This ELISA kit employs a double-antibody sandwich format to detect vaccinia capping enzyme residues. Standards and samples are added to microplate wells pre-coated with an anti-vaccinia capping enzyme antibody. A biotin-labeled detection antibody is added, followed by Streptavidin-HRp, forming an antibody-antigen-biotinylated antibody-Streptavidin-HRP complex.TMB substrate is then added for color development. TMB turns blue upon catalysis by HRP and transitions to yellow after the addition of stop solution. The intensity of the yellow color is directly proportional to the amount of capping enzyme present in the sample.

Principles of Testing

This kit uses double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) method. Add VacciniaCapping Enzyme standard and test samples to the microtiter plate precoated with anti-Vaccinia Capping Enzyme antibody, then add diluted biotin-labeled Vaccinia Capping Enzyme detection antibody, finally add streptavidin-HRP to form the antibody + antigen + antibody-Biotin + SA-HRP complex, wash the plate and add TMB chromogenic solution for color development. TMB is converted from colorless to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The shade of yellow is positively correlated with the amount of Vaccinia Capping Enzyme detected in the sample.

Reagents And Materials Provided

1. Standard, Lyophilized, powder×2vials
2. Coated Plate, 8wells×12 strips
3. Dilution Buffer 1, 45mL×1vial
4. Dilution Buffer 2, 30mL×1vial
5. Wash Buffer(20×), 50 mL×1vial
6. Detection Antibody (100×), 120μL×1tube
7. Streptavidin-HRP(500×), 40μL×1tube
8. TMB Substrate, 15mL×1vial
9. Stop Solution, 10mL×1vial
10. Sealing Film, 4 pieces

Materials Required But Not Supplied

1. ELISA Microplate Reader
2. Microplate incubator/shaker
3. Vortex Mixer
4. Deionized Water
5. Absorbent paper
6. Micropipettes and Pipette Tips

Storage

This kit should be stored at 2-8°C before the kit opening. The kit has a shelf life of 12 months from the date of manufacture. After opening, store the kit at 2-8°C and use within 30 days

Reagent Preparation

1. Equilibrate all reagents and samples to room temperature before use. Record the opening date on the kit. Use opened kits in sequence. Kits of the same lot may be mixed; do not mix different lot numbers. Return unused strip wells to the foil pouch, reseal, and store at 2-8C.

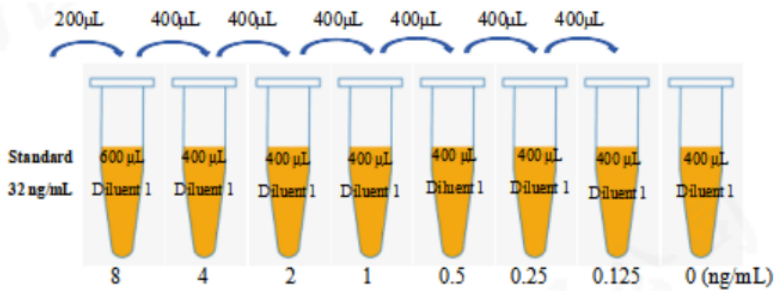
2. 1×Wash Buffer Preparation: Allow 20× Wash Buffer to reach room temperature (18-25°C) and fully dissolve any crystals, mix well. Dilute 1 part of 20× Wash Buffer with 19 parts deionized water. If crystals are present, warm at 50°C water bath until fully dissolved.

3. Standard Curve Preparation: Prepare 8 microcentrifuge tubes labeled with dilution sequence number. Reconstitute 1 vial of lyophilized standard with the indicated volume of Dilution Buffer 1 to obtain a 32 ng/mL stock. Equilibrant 10 minutes. Prepare a 2-fold serial dilution using Dilution Buffer 1 as follows:

Tube 1: 600 uL buffer+ 200 uL of 32 ng/mL standard →8 ng/mL.

Continue 2-fold serial dilutions down to 0.125 ng/mL

The standard dilution details are displayed in the picture below.



4. Testing Sample Preparation: Dilute testing samples with Dilution Buffer 1 to fit within the standard curve range.

5. ERC (Extraction Recovery Control) Preparation: Mix 120 uL of diluted sample with 120 uL of 2 ng/mL standard to obtain the ERC solution.

Assay Procedure

1. Plate Washing 1: Add 300 uL/well of 1XWash Buffer. Discard liquid and tap dry. Repeat 3 times.

2. Sample Incubation1: Add 100 uL/well of standards, samples, and ERC controls. Seal the plate with sealing film and incubate at 37°C for 1 hour. (**Note:** Incomplete sealing may cause evaporation and data deviation)

3. Plate Washing 2: Remove sealing film. Discard liquid. Wash with 300 uL/well of 1× Wash Buffer. Tap dry. Repeat 5 times.(**Note:** pipette above the well surface, avoid touching walls, allow buffer to sit 30 seconds before discarding. Use fresh absorbent paper for drying.)

4. 1×Detection Antibody Preparation: Centrifuge 100× Detection Antibody at 10,000 rpm for 20 seconds. Dilute with Dilution Buffer 2 at 1:99.

5. Detection Antibody Incubation: Add 100 uL/well of 1× Detection Antibody. Seal and incubate at 37°C for 1 hour.

6. Plate Washing 3: Remove sealing film. Discard liquid. Wash with 300 uL/well of 1× Wash Buffer. Tap dry. Repeat 5 times.

7. 1×Streptavidin-HRP Preparation: Centrifuge 500× Streptavidin-HRP at 10,000 rpm for 20 seconds. Dilute with Dilution Buffer 2 at 1:499.

8. Streptavidin-HRP Incubation: Add 100 uL/well of 1XStreptavidin-HRP. Sealand incubate at 37°C for 40 minutes.

9. Plate Washing 4: Remove sealing film. Discard liquid. Wash with 300 uL/well of 1× Wash Buffer. Tap dry. Repeat 5 times.

10. Color Development: Equilibrant TMB substrate to room temperature 10 minutes prior. Add 100 uL/well of TMB, incubate at 37°C in the dark for 15 minutes.

11. Reaction Termination: Add 50 uL/well of Stop Solution. Gently shake to ensure uniform color development.

12. Reading: Within 20 minutes, measure absorbance at 450 nm (reference 630 nm) using a ELISA microplate reader.

Calculation

1. Calculation of Testing Example Residual (vaccinia capping enzyme) Concentration (ng/mL)

   Vaccinia Capping Enzyme Residual of Tesing Sample(ng/mL)

   = Dilution factor

   × Fitting residual Concentration of Testing Sample

2. ERC sample Recovery Rate (%) Calculation:

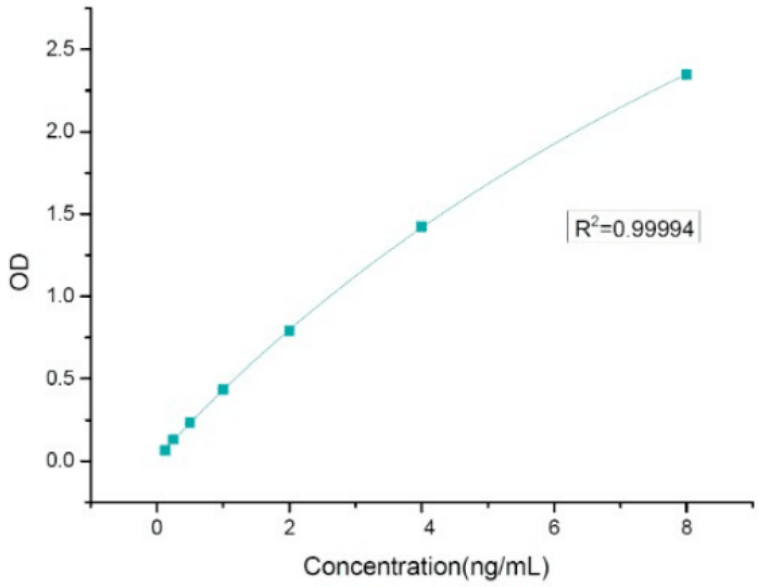
$$ERC\ \ Recovery\ \ Rate = \frac{(ERC\ Sample\ Concentration \times\ Total\ Volume) - (Test\ Sample\ Concentration \times Sample\ volume)}{ERC\ Sample\ \ Th\ \ eoretical\ Concentration \times ERC\ Sample\ \ Volume} \times 100$$

Typical Standard Curve

Standard Curve Fitting: Plot standards' concentrations(x-axis) against OD450 values (y-axis). Apply 4-parameter logistic(4PL) regression for curve fitting.

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
8	2.455	2.374	2.415
4	1.517	1.457	1.487
2	0.886	0.828	0.857
1	0.505	0.492	0.499
0.5	0.310	0.286	0.298
0.25	0.205	0.190	0.198
0.125	0.135	0.128	0.132
0	0.066	0.065	0.066

The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



Detection Range

0.125 - 8 ng/mL

Sensitivity

Limit of quantification: 0.125 ng/mL

Precautions

1. All components in the kit must be equilibration to room temperature(18-25°C) before use.
2. All reagents should be thoroughly mixed before use. Standards should be briefly centrifuged for 5 seconds to collect any liquid adhering to the tube wall or cap. After use, all reagents must be immediately returned to their specified storage temperature.

3. The kit must be used within its expiration date. A standard curve should be prepared for each assay. It is not recommended to mix reagents from different batch numbers.
4. When adding liquids to the ELISA plate, avoid touching the bottom of the wells to prevent damage to the coating. Change reservoirs and pipette tips between different samples and assay steps to avoid cross-contamination.
5. After washing the strip wells, tap them dry carefully to avoid detaching the strips. Do not reuse sealing membranes.
6. A black precipitate may appear during the color development step at high concentrations.This is a normal phenomenon and has no significant impact on final absorbance readings.
7. Ensure the detection wavelength and the chosen fitting equation are correct when reading absorbance values.
8. Optimal assay performance can only be ensured by strictly following the instructions and using all matched reagents provided in this kit.
9. Variability in results may be caused by multiple factors, including operator technique, pipetting method, washing procedure, reaction time or temperature, and storage conditions of the kit.
10. This kit is for research use only and is not intended for clinical diagnostic applications.