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## Rabies Virus ELISA Quantitation Kit (ELISA)

### 【Generic Name】

Rabies Virus ELISA Quantitation Kit (ELISA)

Cat#: 17-0009

### 【Intended Use】

To detect the contents of Rabies Virus (RV) in the samples. It is applicable to the sample detection of RV in vaccine manufacturers and research organizations. It has been successfully evaluated that this kit can detect various serotypes of RVs, including aG, CTN, PV, Flury CVS-11, SRV9, etc.

### 【Principle】

This product adopts the principle of Double Antibody Sandwich Method (Elisa). The flat-bottom 96-well plates are coated with anti-RV antibody. After adding the samples, the anti-RV monoclonal antibody labeled with HRP is used for detection. The content of the RV in the samples can be detected by the degree of TMB color development.

### 【Materials and Reagents】

1. Coated plate, 12 wells × 8 strips
2. Biotin conjugate, 120  $\mu$ L × 1 tube (diluted 100 times for use)
3. HRP-labeled streptavidin
4. BSA, 3G × 1 pack
5. 20× Washing Buffer, 50mL × 1 vial
6. Substrate Solution A, 7mL × 1 vial
7. Substrate Solution B, 7mL × 1 vial
8. Termination Solution, 7mL × 1 vial
9. Sealing plate film, 2 pieces
10. Instruction book

### 【Storage】

1. All components remain stable under the condition of 2-8°C;
2. Avoid light. Valid for six months.

## 【Protocol for Detection】

1. Equilibration  
Equilibrate the required reagents at room temperature (18~25°C) for 30 minutes.
2. Dosing solution: Please configure the reagents before use.
  - 2.1 1× washing buffer: Take 1 vial of 20× washing buffer, dilute it to 1000ml with deionized water, mix well for later use.
  - 2.2 Dilution buffer: Dissolve BSA (3g/pack) completely into 100ml of the prepared 1× washing buffer (step 2.1), mix well for later use.
  - 2.3 Biotin conjugate solution: Take the required Biotin conjugate, dilute it 100 times with the solution buffer prepared in step 2.2, mix well for later use.
  - 2.4 HRP-labeled streptavidin solution: Take the required HRP-labeled streptavidin, dilute it 100 times with the solution buffer prepared in step 2.2, mix well for later use.
3. Adding standard and samples  
Remove the coated plate from the sealed bag and dilute the standard to the different concentrations. After adding 100μl of standard or sample to each well (including negative control), seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes
4. Washing  
Discard the liquid in each well, fill the microwells (350μl/well) with 1× washing buffer, and discard the liquid in the wells after 30 seconds. Repeat these steps for 3 times, then pat the plate on the paper towel after the last wash.
5. Adding biotin conjugate solution  
Add the solution to the microplate (100μl per well). Seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes.
6. Washing  
Repeat step 4.
7. Adding HRP-labeled streptavidin solution  
Add the solution to the microplate (100μl per well). Seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes.
8. Washing  
Repeat step 4.
9. Coloring  
Add 50μl each of Substrate Coloring Solution A and B into each well. Mix well with gentle tapping. Then incubate the plate at room temperature for 2-5 minutes in the dark.
10. Termination  
Terminate the reaction by adding 50μl of 0.2M H<sub>2</sub>SO<sub>4</sub> into each well and mix gently. Set the main wavelength of the microplate reader at 450nm and the reference wavelength at 630nm. Measure the absorbance (OD value) of each well.

## 【Result estimate】

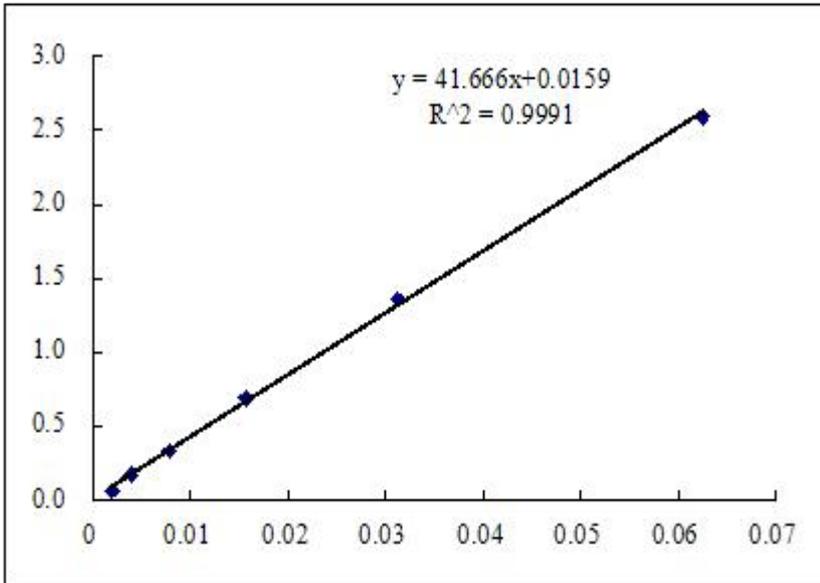
Cut off value=2.1 × N (N is A<sub>450</sub> for negative control. set N=0.05, if N<0.05)

If A<sub>450</sub> for negative control is larger than 0.15, this assay is failed.

If sample A<sub>450</sub> is smaller than cut off value, this sample is negative. And vice versa.

### 【Product Performance Index】

1. Linear range: 1:80~1:640
2. Sensitivity: 1:1280
3. Accuracy: CV%  $\leq$ 15% (n=10)



### 【Limitations】

1. This kit is only used to detect the content of RV in samples.
2. Results out of the measurement range of the kit are unreliable.
3. Severe hemolysis, chyle, and bilirubin samples may cause abnormal test results.
4. This kit is developed for in vitro research only

### 【Caution】

1. Avoid cross contamination.
2. Follow reader measure as a standard.
3. All samples and buffers should be added or removed with pipette.
4. Do not mix reagents from different batches.