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AK200-Mouse Rabies Virus Protective Antibody Detection Kit

Instructions for AK200 ELISA Kit

【Purpose】

The AK200 ELISA Kit is designed for semi-quantitative detection of rabies virus protective antibody in mouse serum samples, for in vitro research use only.

Cat# AK200M1

【Experimental principle】

Briefly, flat-bottom 96-well plates are coated with Glycoprotein of rabies virus that captures rabies virus protective antibody in the sample. A quick wash removes any unbound particles. Captured rabies virus protective antibody is detected by Goat Anti-Mouse IgG pAb, which is conjugated to horseradish peroxidase (HRP). Finally, the chromogenic substrate 3, 3´, 5, 5´-tetramethylbenzidine (TMB) is added. The amount of rabies virus protective antibody is proportional to the color generated in the coupled oxidation-reduction reaction compared with known amounts of standards.

【Kit Components】

96-well plate coated with Glycoprotein, 8 wells×12 strips

Negative control, 40μL/tube

Positive control, 40μL/tube

Sample Dilution Buffer, 12mL

Goat-anti-Mouse IgG pAb (HRP Conjugated pAb, 100X), 120μL

BSA, 3g

20X PBS Buffer, 50mL

Substrate Solution A, 6mL

Substrate Solution B, 6mL

Stop buffer, 6mL

Sealing films, 2 pieces

Assay Instruction

【Storage Conditions】

When stored at 2~8°C, the product is stable for one year.

【Protocol】

A. Before using, pre-warm all the reagents to room temperature (18~25℃).

B. **Washing buffer** dilution: dilute 20X PBS Buffer to 1000mL and mix well.

C. **Enzyme Dilution buffer (for HRP conjugated pAb)**: dissolve 3g BSA to 100mL washing buffer and mix well.

D. Dilute samples with **Sample Dilution Buffer** and add 100μL into each well of the ELISA plate. Set negative control, positive control and incubate at 37℃, 200 rpm for 60min.

E. Remove samples from wells and wash all wells three times with prepared Washing Buffer. Remove residual solution with paper pat.

F. Dilute 100μL Goat-anti-Mouse IgG pAb with 10mL **Enzyme Dilution buffer (for HRP conjugated pAb)** and mix well. Add 100μL of diluted Goat-anti-Mouse IgG pAb to each well. (Careful not to touch or scratch the surface of the wells). Incubate plate at 37℃, 200rpm for 60min.

G. Remove samples from wells and wash all wells three times with prepared Washing Buffer. Remove residual solution with paper pat.

H. Add 50μL each of Substrate solution A and Substrate solution B into each well. Mix thoroughly with shaking. Incubate at 25℃ for 5 min and avoid light.

I. Stop the reaction by adding 50μL of Stop Solution.

J. Record the absorbance at 450nm on a plate reader within 30min.

【Properties】

1. Precision: coefficient of variation CV (Percentage) should not be higher than 15 % (n=10).

2. Stability: The components of the kit are left for 7 days at 37℃ under accelerated destruction condition, and all performance indexes still meet the above requirements.

【Caution】

A. Avoid cross contamination.
B. Follow reader measure as standard.
C. All samples and buffers are added or removed with pipette.
D. Do not mix reagents from different batches.