VERSION 1.01

RABIES VIRUS ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Rabies Virus Antibody ELISA kit For Dogs and Cats

Catalogue Number. IP100197

Introduction

The Rabies Virus (RBV) antibody ELISA kit is used to test rabies virus antibody in serum of dogs, cats etc., used to assess the status of rabies vaccination.
This kit use block ELISA method, rabies antigen is pre-coated on enzyme micro-well strips. When testing, add diluted serum sample, after incubation, if there is rabies virus specific antibody, it will combine with the pre-coated antigen, discard the uncombined antibody and other components with washing; then add enzyme labled anti-rabies virus monoclonal antibody, antibody in sample block the combination of monoclonal antibody and pre-coated antigen; discard the uncombined enzyme conjugate with washing; Add TMB substrate in micro-wells, the blue signal by Enzyme catalysis is in inverse proportion of antibody content in sample, use ELISA reader at 450nm wavelenth to measure the absorbance A value in reaction wells after adding stop solution to stop the reaction.

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	RBV-Ag Coated plates	1/2 plate of 96 wells
2	Enzyme Conjugate	11/22 ml
3	10X Concentrated washing buffer	100 ml
4	Substrate	11/22 ml
5	Sample diluent	100 ml
6	Stop solution	11 ml
7	Negative control	1/2 ml
8	Positive control	0.5/1 ml
9	Adhesive Foil	2/4 pieces
10	Instruction sheet	1 piece

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader
5. Distilled water or deionized water

6. Bottle or microplate washing machine

Sample preparation

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

Washing buffer preparation

Return 10X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can store at 4℃ for about 1 week.

Sample dilution

At serum dilution plate, dilute serum at 1:100 with sample diluent.
Notice: Negative control and Positive control do not need dilute. Exchange tip after taking sample every time, record the situation of the sample on plate accurately. Shake the sample evenly before adding it

Operation procedures

1 .Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 µL/well. Others are wells for samples, add 100µL/well of Sample diluent solution, then add 1 µL serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37℃ for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution(dillute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 µL enzyme comjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 ℃ for 30 min.
6. Repeat step 3(washing).
7. Add 50µL substrate A and substrate 50µL B into each well, mix properly, incubate for 15 min at 37 ℃ in the dark with new adhesive foil.
8. Add 50µL stop solution into each well, mix gently and determine the result within 5-30 min.
9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

Results judgment

Read the OD value at 450nm (630nm as reference).
For the assay to be valid:
OD value of negative control(N) > 0.5, meanwhile positive value (P) blocking rate > 60%

Calculate method:

PI(blocking rate)= 1- (Sample OD value/ Negative control OD average value)

Results interpretation

PI(blocking rate)> 60%: Positive
PI(blocking rate)≤ 60%: Negative

(Note: when PI=60%, it means antibody titer 0.5 IU/ml)

Notes

1. Return all reagents into room temperature before use, shake it evenly before use, and store back to 2-8℃ after usage.
2) Do not mix use reagents from different kits and different lot no., prevent the reagents been polluted when using.
3) Substrate A and stop solution may have irritation to skin and eyes, be careful to use.
4) Do not expose Substrate to strong light and avoid contact with the oxidant.
5) RBV-Ag coated plates should be sealed and moisture-proof. Put back unused MicroWell plate into dry foil bag and sealed at 2~8 ℃.
6) All wastes should be treated well to avoid pullution before discarding.
7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.
8) RBV-Ag coated plates is disposable, do not repeat use.

storage: store at 2-8℃, dark, sealed, dry place, no frozen..
Expiry date: 12 months; date of production is on box.