Rabies virus (RABV) Glycoprotein (G) ELISA Kit (VK584018)

MATERIALS PROVIDED & STORAGE CONDITIONS

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Sample Diluent	50 mL
Stop Solution	6 mL
Positive Control	0.8 mL
Negative Control	0.8 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

* Provided this is within the expiration date of the kit.

Test principle

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Microtiter wells are coated with rabies virus antibody, which captures rabies virus G protein in the sample. After addition of HRP-labelled rabies virus antibody, a “coated antibody – antigen – enzyme-labelled antibody” complex is formed. Upon addition of chromogen substrate, the HRP conjugated to the complex converts the substrate into a blue product, which turns yellow after the stop solution is added. The absorbance (A) is measured with a microplate reader. A double-logarithmic standard curve is fitted using the concentration values of the standards and their corresponding absorbance values, and the relative content of rabies virus G protein in the sample is calculated from the curve.

Other materials required but not supplied

Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transferpettor, EP tubes and disposable pipette tips

Deionized or distilled water

Absorbent paper

Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to s trong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the microplate reader.
8. **Each reagent is optimized for use in the VK584018. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other VK584018 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample Requirements

1. This kit is intended for use with finished rabies vaccines and in-process intermediates. Other sample types are not suitable.
2. Samples should be free from microbial contamination. Samples may be diluted with 0.02 M phosphate-buffered saline (PBS, pH 7.4). A starting dilution of 1:5 is recommended; the dilution ratio should be adjusted according to the expected concentration. Diluted samples should be used promptly and not stored.
3. Allow samples to equilibrate to room temperature for at least 30 minutes before use. Frozen samples must be thoroughly mixed before testing.
4. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Equilibration:** Allow the kit to reach room temperature (about 30 minutes).
2. **Wash buffer preparation:** Dilute the concentrated wash buffer (20×) 1:20 with distilled or deionized water.
3. **Dilution:** Dilute the standards and test samples as required. If using WHO standards for the standard curve, a starting concentration of 0.05 IU/mL is recommended, with at least 4 serial dilutions.
4. **Addition of samples:** Add 100 µL of each test sample, standard, etc., to the respective wells. Include one blank well (if using dual-wavelength measurement, a blank well may be omitted).
5. **Incubation:** Seal the plate with the sealing film and incubate at 37 °C for 30 minutes.
6. **Washing:** Carefully remove the sealing film and wash the plate 5 times with the plate washer. After the final wash, tap the plate dry as much as possible.
7. **Addition of enzyme conjugate:** Add 100 µL of enzyme conjugate reagent to each well, except the blank well.

8. **Incubation:** Repeat step 5.
9. **Washing:** Repeat step 6.
10. **Colour development:** Add 50 µL of Chromogen Solution A and 50 µL of Chromogen Solution B to each well. Gently mix by swirling, and incubate at 37 °C in the dark for 15 minutes.
11. **Measurement:** Add 50 µL of Stop Solution to each well, mix gently, and read the absorbance within 10 minutes. Set the microplate reader to 450 nm (dual-wavelength reading at 450 nm/600–650 nm is recommended). Zero the reader using the blank well and read the absorbance (A) of each well.

Interpretation of the results

1. Construct a double-logarithmic standard curve using the antigen concentrations of the standards (X-axis) and their corresponding absorbance values (Y-axis). Derive the linear regression equation. Calculate the antigen content in the sample by substituting the logarithmic absorbance value of the sample into the regression equation.
2. Example using natural logarithms (LN): use the LN of the standard antigen concentrations as the independent variable (X) and the LN of the corresponding absorbance values as the dependent variable (Y) to obtain the linear regression equation. The LN of the sample absorbance is then used to calculate the antigen content.
3. The absorbance of test samples should ideally be within 0.1–2.0. If outside this range, adjust the dilution ratio. The measured value of the test sample must fall within the range of the standard curve; otherwise, accurate quantification is not possible.
4. The assay is considered invalid and must be repeated if any of the following occur:

   Correlation coefficient (r) < 0.99

Limitations of this test method

1. **For research use only.** Operate strictly in accordance with this instruction manual. Users must receive professional training. Wear gloves and laboratory coats during the procedure.
2. Sealing films are not reusable. Microtiter plates and enzyme conjugate reagents from different lots must not be interchanged, nor mixed with reagents from other manufacturers.

3. Avoid working in environments containing volatile substances or hypochlorite disinfectants (e.g., bleach).

4. Allow the kit to equilibrate to room temperature (about 30 minutes) before use. Gently vortex reagents before use and return them to 2 – 8 °C immediately after use. Return unused microtiter strips to the resealable pouch with desiccant and store at 2 – 8 °C. Do not use expired reagents. Avoid microbial contamination of reagents.

5. Use a calibrated pipette for all additions. Change pipette tips and reagent reservoirs when switching between different samples or reagents to prevent cross-contamination.

6. During washing, ensure each well is completely filled with wash buffer to remove unbound enzyme. If using an automatic plate washer, set a soaking time of 30–60 seconds. After washing, proceed immediately to the next step; do not allow the plate to dry out. Avoid prolonged interruptions to ensure uniform conditions across all wells.

7. Results must be read using a microplate reader. Wipe the bottom of the plate clean before reading, and ensure there are no air bubbles in the wells. Do not touch the bottom outer surface of the wells, as fingerprints or scratches may affect the reading.

8. Critical instruments should be regularly calibrated and maintained.