Kalon Biological Ltd

RHDV antibody EIA

An enzyme immunoassay to detect antibodies to rabbit haemorrhagic disease virus in rabbit serum.

INTRODUCTION

The highly contagious rabbit haemorrhagic disease virus (RHDV) causes a lethal infection of both domestic and wild European rabbits (*Oryctolagus cuniculus*). The disease was first observed in China and has since been found in many wild rabbit populations across the world. The positive stranded viral RNA is enclosed in a capsid protein termed VP60 which forms the basis of the test.

PRINCIPLE OF THE ASSAY

The assay is based on the indirect ELISA principle.

Polystyrene microtitre plate wells are supplied pre-coated with recombinant RHDV antigen. Serum samples are diluted and incubated in these wells, during which reactive antibody is captured. After a wash step the surface is probed for antibodies by incubation with a peroxidase conjugated anti-rabbit immunoglobulin tracer. Following a second wash step, enzyme substrate and a chromogenic reagent are added to the wells. The enzyme incubation is halted by the addition of acid, which also has the effect of both changing and enhancing the amount of colour produced. This is measured in a photometer. The optical density is proportional to the amount of RHDV antibody present in the original sample. This is compared with a cut-off calibrator that has been designed to distinguish between non-specific binding and a true positive reaction and is run in each assay.

KIT PRESENTATION

❑	Antigen coated microwell plate	96 wells
❑	Positive Control, pre-diluted	1 mL
❑	Cut-off Calibrator, pre-diluted	2 mL
❑	Negative Control, pre-diluted	1 mL
❑	Tracer Concentrate (×41)	0.5 mL
❑	Assay Diluent	120 mL
❑	Wash Concentrate (×40)	50 mL
❑	HRP Substrate Solution	15 mL
❑	TMB Chromogen	0.5 mL
❑	Stop Solution (0.5 mol/L sulphuric acid)	15 mL
❑	Resealable plastic bags	2

Store the reagents refrigerated.

ADDITIONAL REQUIREMENTS

- ❑ Disposable tip micropipettes to deliver volumes from 10 µL to 1 mL, preferably multichannel pipettes for 10 and 200 µL.
- ❑ Clean volumetric laboratory plastic or glassware.
- ❑ Washer bottle or automatic microtitre plate washer.
- ❑ Disposable paper towels.
- ❑ Microtitre plate photometer fitted with at **450 nm** filter.

SAFETY PRECAUTIONS

Treat all serum and plasma samples as potentially infectious.

ASSAY PROTOCOL

Preparation

1) Prepare a 1 in 101 dilution of each specimen to be tested by adding 10 µL sample to 1 mL Assay Diluent. Mix thoroughly before use. *Do not dilute the kit controls*; they are supplied to you pre-diluted.

2) Prepare the required volume of working strength tracer by mixing Tracer Concentrate and Assay Diluent 1 part to 40. 1 mL is required per 8-well strip, so for example add 300 µL Tracer Concentrate to 12 mL Assay Diluent for a whole plate.

3) Prepare the required volume of working strength wash solution by diluting Wash Concentrate 1 in 40, e.g. make 25 mL Wash Concentrate to 1 L with purified water.

4) Prepare the required volume of working substrate solution by mixing TMB Chromogen and Substrate Solution 1 part to 40; for example add 300 µL TMB Chromogen to 12 mL Substrate Solution for a whole plate. The substrate solution made in this way is colour coded pink as an aid to dispensing. A blue colour prior to use indicates that the solution is contaminated. In this circumstance, a fresh dilution into clean glassware should be made.

Primary Incubation

5) Dispense 100 µL of each kit calibrator/control into designated microwells, Positive Control, Cut-off Calibrator - in duplicate - and Negative Control.

6) Dispense 100 µL of each diluted specimen into designated microwells

7) Seal the plate in the plastic bag and incubate at 37°C for 30 minutes.

8) Wash the microwells -

Alternately fill and aspirate the microtitre wells with 350 µL of fresh working strength wash solution a total of four times. Tap out residual wash solution on paper towelling ready for the next step.

Secondary Incubation

9) Dispense 100 µL working strength Tracer

10) Seal the plate in the plastic bag and incubate at 37°C for 30 minutes

11) Wash the microwells as before.

Enzyme Incubation

12) Dispense 100 µL working substrate solution

13) Incubate the plate uncovered at 18° to 25°C for 30 minutes

14) Dispense 100 µL Stop Solution into each microwell.

Assay Completion

15) Read the microwell optical densities at 450 nm within 30 minutes of adding Stop Solution. The microtitre plate reader should be blanked on air or using a 620 nm reading.

16) Verify the assay.

The assay can be considered valid if the protocol has been followed correctly, the Positive Control optical density is greater that 0.8, the Negative Control is less than 0.25, and the ratio of the Cut-off Calibrator to the Negative Control is at least 2.0.

17) Interpret the results.

Score results with an optical density greater than Cut-off × 1.1 as positive

Score results with an optical density less than Cut-off × 0.9 as negative

Results between these values, that is 0.9 ≤ Cut-off ≤ 1.1, are equivocal and should be repeated to confirm the status.

EXPECTED RESULTS

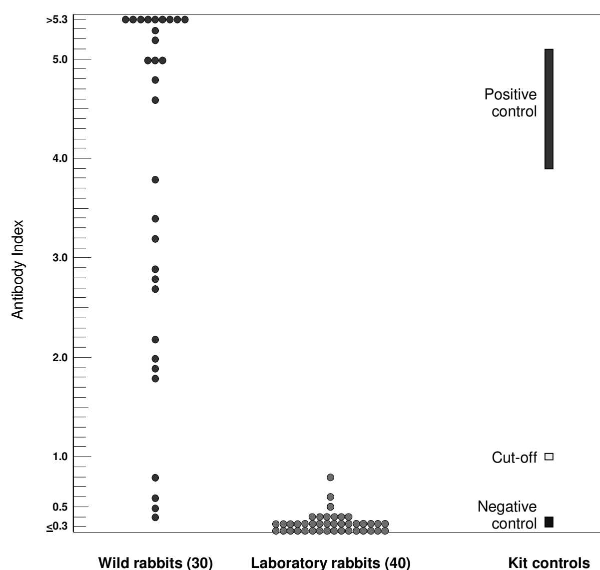
Performance

Serum samples from 30 wild rabbits and 40 laboratory-bred rabbits were screened for the presence of antibodies to RHDV. 36/40 (90%) of the wild rabbits and 0/40 (0%) of the laboratory rabbits were above the cut-off calibrator (see graph).

Prevalence

RHDV is not expected in laboratory-bred rabbits. The prevalence in wild populations will vary greatly with geographical location. Evidence exists for the presence of avirulent strains in some populations.

As with all serological tests, negative result does not preclude the possibility of a very recent infection: a susceptible animal may die before the appearance of an immune response.



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