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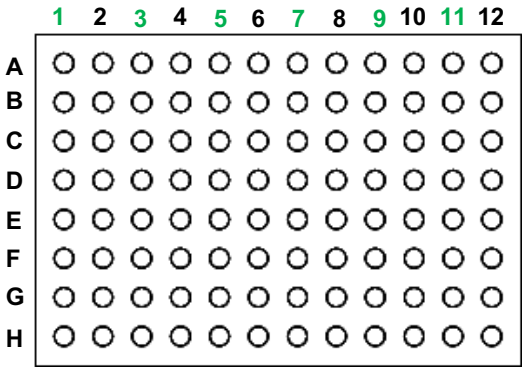
User Manual: ELISA Kit for the Direct Detection of Antibodies Against Kilham's Rat Virus and Toolan's H-1 Virus in Mouse Serum Samples – 96 wells

Upon receipt store at 4 C for short term storage (≤ 2 weeks). For longer term storage, plates and antibody controls can be stored at -20 C or colder. Do NOT freeze other reagents. Do NOT repeatedly freeze & thaw antibody controls.

Principle

When diluted sera are added to the test plate, antibodies that are reactive with the antigen or control antigen will bind to coated wells. After washing to remove non-bound elements of the sample, the conjugate is added. If antibodies have been bound to the wells of the plate, the conjugate will bind to these antibodies. After the conjugate incubation, another series of washes removes unbound material, and then a chromogenic substrate is added. If the conjugate is present, the peroxidase from the chromogen will catalyze a reaction that turns the chromogen from clear to blue-green (if using ABTS chromogen substrate). The plate reaction is read when the OD value of the positive control reaches the desired OD value. The Net OD (Antigen well OD - Control Antigen OD) of the specimen is used to evaluate results. See "Evaluating Results" section.



Antigen is coated in odd numbered columns (1, 3, 5, 7, 9, 11)

Control Antigen is coated in even numbered columns (2, 4, 6, 8, 10, 12)

Note: The plates are provided in strip well format (12 removable strips per plate), to allow for increased experimental flexibility, efficient use of reagents, and minimization of cross-contamination.

Note: The kit contains all the reagents required to run the ELISA test, ready to use.

Sample Preparation

1. Collect 25-50 µL of blood from the animal.
2. Let the sample stand for 30 min.
3. Spin the blood at 2,000 – 3,000 rpm (1,000 – 2,000 x g) for 10 minutes and collect the serum (top layer) in a new tube.

Recommended Procedure

1. Dilute **10X Wash Solution-Concentrate** to 1X Concentration by a 1:10 dilution with DiH2O. Example: 1 mL of 10X Wash Solution to 9 mL of DiH2O.
2. Wash plate by adding 100 L of 1X Wash Solution and discarding solution. Repeat this step three times. Blot dry on a paper towel prior to use.
3. Prepare **Working Milk Diluent** by adding one vial of 0.5 g **Milk Powder** into the 10 mL **Milk Diluent** bottle. Transfer remaining powder by adding 1-2 mL of working **Milk Diluent** to **Milk Powder** vial; pipette up and down and transfer. Mix until powder is dissolved.
4. Dilute **Positive Control** antibody to 1:2 dilution with working **Milk Diluent** by adding 50 µL of the **positive control** and 50 µL working **Milk Diluent** to appropriate antigen well(s) and adjacent control antigen well(s).
5. Dilute **Negative Control** antibody to 1:2 dilution with working **Milk Diluent**. Add 50 µL of the **negative control** and 50 µL **Milk Diluent** to the appropriate antigen well(s) and adjacent control antigen well(s).
6. Prepare 1:50 dilution of test sample(s) in working **Milk Diluent** by adding 4.2 µL of serum in 205 µL of Milk Diluent. Add 100 µL of diluted samples to appropriate antigen well(s) and adjacent control antigen well(s).
7. Incubate the plate, covered, at 37˚C for 60 – 65 minutes.
8. Wash plate three times with 100 µL of **1X Wash Solution** and blot dry on a paper towel.
9. Add 100 µL per well of **species-specific conjugate antibody**.
10. Incubate the plate, covered, at 37˚C for 60 – 65 minutes.

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11. Wash plate three times with 100 L of **1X Wash Solution** and blot dry on a paper towel.

12. Add 100 µL per well of **ABTS® Peroxidase Substrate**.

13. Incubate the plate, covered, at 37°C for 30 – 35 minutes.

14. If the plate will be tested immediately after final incubation:

    a. Do NOT add Stop Solution.

    b. Read the plate@405 nm.

    c. If the positive control does not meet the minimum OD upon initial reading (see below), continue incubation of plate for an additional 5-15 minutes, then re-read the plate.

15. If the plate will NOT be tested immediately after final incubation:

    a) Add 100 µL of **1X Stop Solution** immediately after final incubation.

    b) Read the plate@405nm within 45 minutes of adding the stop solution.

Evaluating Results

Net OD= Antigen – Control Antigen well

Positive control: Net O.D value should be ≥**0.8 OD units.**
Negative control: Net O.D value should be <**0.30 OD.**
Samples with net O.D value ≥ **0.30** are considered reactive.
Samples with net O.D value < **0.30** are considered negative.

Recommendations

•Once prepared, the working Milk Diluent can be used for up to 7 days if refrigerated. Working milk diluent can also be frozen prior to use.

•Do not pipette ABTS® Peroxidase Substrate straight from the bottle or pour substrate back into the bottle if pipette tip has touched an antigen well and then the substrate. It will cause the substrate to catalyze and present false positives in future use.

•Do not scrape the bottom of the ELISA plate with pipette tip. This will remove antigen coating and create false negatives.

Items Included

User Manual
2 ELISA Plates (48 tests per plate)
1 mL Positive Control Antibody
1 mL Negative Control Antibody
20 mL 10X Wash Solution - Concentrate
2 X 10 mL Milk Diluent
2 X 0.5 g Milk Powder
20 mL species-specific conjugate antibody, Peroxidase-Labeled.
20 mL ABTS® Peroxidase Substrate
20 mL 1X Stop Solution