Bovine Japanese Encephalitis Antibody (IgG) Elisa Kit

SKU: EK760308

Product Information

- Assay Type: Qualitative
- Species: Bovine
- Sample Type: serum , plasma , urine , tissue homogenates , cell culture supernates
- Size: 96 Wells

Components

Assay plate (12 × 8 coated Microwells)	1
Positive Control	1×0.5ml
Negative Control	1×1.5ml
HRP-Conjugate Reagent	1×6ml
Sample Diluent	1×6ml
Chromogen Solution A	1×6ml
Chromogen Solution B	1×6ml
Stop Solution	1×6ml
Wash Solution	1×20ml×30 fold
User manual	1
Adhesive Strip	1

Test Principle

The ELISA is based on the the Qualitative immunoassay technique. The Microplate provided in this kit has been pre-coated with an Antibody specific to Japanese Encephalitis Antibody, make it to solid-phase antibody. Samples are added to the Microplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for Japanese Encephalitis Antibody is added to each Microplate well and incubated, so the antibody-antigen-Enzyme labeled antibody complex is formed. Following a wash to remove any unbound reagent, then the TMB substrate solution is added to each well. Only those wells that contain Japanese Encephalitis Antibody and HRP-conjugated Japanese Encephalitis Antibody antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The qualitative determination of Japanese Encephalitis Antibody is determined by comparing with the CUTOFF value.

Sample Collection

Serum - Use a serum separator tube and allow samples to clot for two hours at

room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (Different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v = 1:20-1:50, e.g. 1mL lysis buffer is added in 20-50mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10,000×g. Collect the supernates and assay immediately or aliquot and store at ≤-20°C.

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS
3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤ -20°C.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8°Cfor15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Feces - Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg feces), sonicated(or mashed)and centrifuged at 5000×g for 10 minutes, where the supernatant was collected for testing.

Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

Reagent Preparation

Prepare wash buffer | Combine 20 ml concentrated wash buffer (whole bottle) with 580 ml distilled water.

Assay Procedure

1. Set a blank well without any solution.
2. Pipette Positive control and Negative control 50 ul to the well. Controls need test in duplicate. Pipette sample diluent 40 ul and testing sample 10 ul to testing sample well. Pipette sample to the bottom of well, don’t touch the wall as far as possible, and mix slowly and carefully.
3. Cover with the adhesive strip, and then incubate for 40 min at 37℃.
4. Dilute wash solution 30-fold with distilled water
5. Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 30s then drain, repeat this 5 times.
6. Pipette HRP-Conjugate reagent 50 ul to each well, except blank well
7. Incubation as described in Step 3
8. Washing as described in Step 5
9. For coloring; add 50 ul Chromogen Solution A and 50 ul Chromogen Solution B to each well, mix slowly and carefully shake and incubate at 37℃ for 20 minutes. Please avoid light during coloring.
10. For termination; add 50 ul stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Calculation of Results

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative result : if the OD value

Positive result: if the OD value ≥ CUT OFF, the sample is positive.

Storage

Store kit at 2-8ºC immediately upon receipt. For long term the whole kit could be stored at -20 ºC in shelf life.

Download our ELISA technical hints, troubleshooting tips
www.afgsci.com/troubleshooting/

For technical support contact information, visit: https://www.afgsci.com/contact/