ELISA Procedure

1. Take out the required # of strips or the whole coated plates and record the sample position on a worksheet. Dilute the negative and positive control serum at 1:3. Dilute sample 1:40 with sample diluent. Prepare 1x Wash buffer by diluting 1:20 with water.

2. **Add 100 ul of sample diluent to 1 well (blanks) and diluted negative, positive controls (supplied in the kit) and samples (diluted 1:40) in duplicate**. Mix gently 5-10 secs manually, **incubate at 37oC for 30 min**. If 37oC incubator is not available, incubation can be performed at room temp (25-28oC) for 60 min.

3. **Wash each well with 300 µL washing buffer 4 times**. After the last wash, tap the plates on a paper towels/absorbent paper to remove traces of wash buffer. We recommend using an automated ELISA washer.

4. **Add 100 µL enzyme conjugate into each well**. Mix gently for 5-10 secs manually and **incubate at room temp for 30 min** (or room temp for 45 min).

5. **Wash each well with 300 µL washing buffer 5 times**. After the last wash, tap the plates on a paper towels/absorbent paper to remove traces of wash buffer.

6. **Add 100 ul of TMB substrate into each well**.. Mix gently for 5-10 seconds. Cover the plate with aluminum foil or paper and **incubate for 10-15 min at room temperature** (incubation time can be adjusted so as to produce better background (blanks) to sample ratio). Color (blue) develops in positive wells.

7. Add **stop solution (50 µL) to each well**. Blue color turns yellow. Read the plate at 450 nm within 10 min. It is also possible to read the plates at dual wavelength of 450nm and reference at 630nm.

CALCULATION OF RESULTS

Set zero for the blank well, and test the OD450/630 value on the ELISA reader. For the assay to be valid, the supplied positive control must be >0.4 and the negative controls ≤0.2. .

Positive Samples values >0.4 (or 2X the negative controls)
Negative **Samples** values <0-0.2
Equivocal values sample values between 0.2-0.4 are ambiguous and doubtful. Repeat the test or take samples at different time point.

Specificity and species Crossreactivity

This kit uses anti-Pig IgG-HRP conjugate that will only detect IgG type antibody to the Encephalitis B (JEV) Virus, IgM and IgA subtype will not be detected. It is possible to use anti-Pig IgM/IgA antibody HRP conjugates to detect these antibodies., This kit is designed for pig sample and it will not be suitable for other species (e.g. human etc).

ELISA kits available from us:

AE-200100-2	Swine/Porcine Toxoplasmosis IgG Antibody ELISA kit, 2x96 tests
AE-200120-2	Swine Foot and Mouth Disease virus (FMDV) antibody ELISA kit,
AE-200125-2	Swine Foot and Mouth Disease virus (FMDV) IgG Distinguishing kit,
AE-200130-2	Swine/Porcine Pseudorabies Antibody ELISA kit, Quantitative, 96 tests
AE-200135-2	Swine/Porcine Pseudorabies Virus IgE Antibody Distinguishing kit,
AE-200140-2	Hog (Swine/Porcine) Cholera Virus Antibody ELISA kit, 2x96 tests
AE-200150-2	Swine/Porcine Circovirus ELISA kit, 2x96 tests
AE-200170-2	Swine/Porcine Parvovirus (PV) Antibody ELISA kit, 2x96 tests
AE-200180-2	PRRSV antibody ELISA kit, 2x96 tests

Instruction Manual No. AE-200160

Swine/Porcine Encephalitis B Virus ELISA KIT

Cat. #AE-200160-2 (96 wells x2)

For detection of Encephalitis B (JEV) Virus antibodies in Porcine/Swine/Pig serum.

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE COSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Porcine Encephalitis B Virus (JEV) ELISA test
KIT Cat #AE-200160 (192 tests)

Kit Components	200160-2 96x2
Porcine Encephalitis virus Antigen coated microtiter plate, #200161	2 plates
Negative serum #200162N	1.5 ml
Positive serum, #200162P	1.5 ml
Sample diluent solution #200160SD	50 ml
Anti-Pig IgG-HRP conjugate # 200163	22 ml
20x concentrated washing buffer 200160WB	30 ml
TMB Substrate solution #200160SA	22 ml
Stop solution #200160ST	12 ml
Instruction Manual #M-AE-200160	1

INTRODUCTION

Porcine Encephalitis Virus is also known as Japanese Encephalitis virus (JEV). Japanese encephalitis virus is the virus responsible for the Japanese B encephalitis disease. JEV is a positive single stranded enveloped RNA virus that belongs to the Flaviviridae family from the genus Flavivirus. JEV is called arbovirus because it is transmitted by the Culex tritaeniorhynchus mosquitoes. The main reservoir of JEV is the pigs, and once transmitted to human it can cause severe symptoms. The pigs infected by the virus shows no symptoms except in pregnant ones which will lead to miscarriage or abnormal fetus.

Human can get infected with the virus by the Culex mosquitoes. Mosquitoes will become infected when they fed themselves with infected pigs, those mosquitoes now can infect human. This virus cannot be transferred from human to human or pigs to human, only from mosquitoes to humans. Once the virus enters the human body it follows an incubation period of four to fourteen days. The symptoms will start with fever and headache, however it can progress giving worse symptoms such as neck stiffness, stupor, disorientation, coma, tremors, occasional convulsions and spastic paralysis.

Alpha Diagnostic Intl's Porcine Encephalitis B virus (JEV) ELISA Test kit is a highly sensitive indirect type assay for the detection of encephalitis virus IgG antibodies in swine/pig serum. This kit can be used to assess the immunity conditions against porcine Encephalitis B virus in the pig farms.

The ELISA Test Kit is for research use only.

PRINCIPLE OF THE TEST

The kit is based on an indirect enzymatic immunoassay (Indirect ELISA). The antigen is coated on plates. When a sample serum contains specific antibodies against virus, they will bind to the antigen on plates. Wash the unbound antibodies and other components. Then add a specific peroxidase conjugate (IgG-HRP). After incubation and washing, add the TMB substrate. A colorimetric reaction will appear, measured by a spectrophotometer (450 nm). Intensity of the color is directly proportional to the amount of the antibodies. Results are compared with the supplied negative and positive controls.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: Constant temperature box or incubator, microtiter plate spectrophotometer (450/630 nm) and absorbent paper

Micropipettors: 50 µL, 100 µL and 1000 µL

PRECAUTIONS AND SAFETY INSTRUCTIONS

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained.

SAMPLE PRE-TREATMENT

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
2. Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment

Washing buffer: dilute 20×concentrated washing buffer with deionized water (30 ml stock and 570 ml distilled water). Store 1x wash buffer at 4oC.