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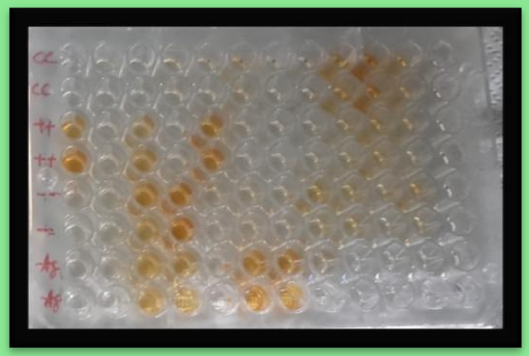
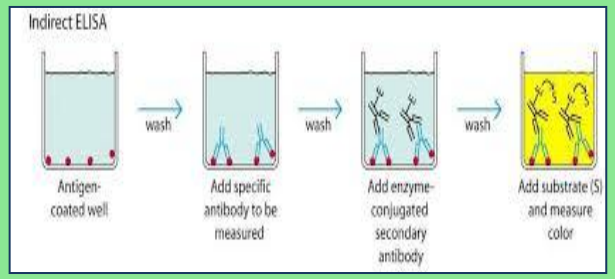
DEPARTMENT OF BIOTECHNOLOGY

# *Single dilution indirect ELISA kit for detection of Classical Swine Fever virus antibody*







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*The Indirect ELISA test is based on the principle of binding of known antigen with the specific antibody present in the test sera. The present kit is based on the diagnostic efficiency of purified swine fever virus for sero-diagnosis of classical swine fever.*

## *REAGENTS PROVIDED IN THIS KIT*

| *SL No.* | *Reagents* | | *Quantity* | *Storage* |
|---|---|---|---|---|
| 1 | *Phosphate buffer saline* | Prepare PBST | *For 50 reactions* | *4⁰C* |
| 2 | *Tween 20* | | | |
| 3 | *Carbonate bicarbonate buffer* | | | *4⁰C* |
| 4 | *OPD* | | | *-20 ⁰C* |
| 5 | *Lactalbumin hydrolysate* | | | *4⁰C* |
| 6 | *Stopping solution 1M $H_2SO_4$* | | | *Room temperature* |
| 7 | *Anti-pig conjugate* | | | *-20 ⁰C* |
| 8 | *Purified CSFV antigen* | | | *-20 ⁰C* |
| 9 | *Standard positive serum* | | | *-20 ⁰C* |
| 10 | *Standard negative serum* | | | *-20 ⁰C* |

***Reagents are supplied in vials for 50 reactions.***

***COATING BUFFER (Carbonate-bicarbonate buffer):*** *Dissolve the total content of the capsule in 100 ml of sterile distilled water and prepare aliquots of 10 ml and store at -20⁰C*

***Coating antigen:*** *Cell cultured purified antigen reconstituted in Carbonate Bicarbonate buffer with a final concentration 10µg per well.*

***Washing buffer (PBS-T):*** *Dissolve a sachet of PBS and Tween 20 in 500ml of distilled water to prepare PBST.*

***BLOCKING BUFFER:*** *Prepare 20 ml (for one plate) of blocking buffer containing 1 gm Lactalbumin hydrolysate in 20 ml of PBST (wash buffer)*

***Chromogen-substrate solution:*** *One OPD tablet set is used for the preparation of 20 ml chromogen-substrate solution. Dissolve one buffer tablet in 20 ml of distilled water completely and then add the OPD tablet subsequently. The substrate solution may be stored as 6 ml aliquots in -20⁰C for further use. In case colour appears in the solution vial should be discarded.*

***Stopping solution****: 1 M $H_2SO_4$*

## *TEST PROTOCOL*

*1. Dispense 50µl of diluted antigen in carbonate bicarbonate buffer (Ph 9.6) to all the wells of ELISA plates (except the C1-G&H as antigen blank well) and incubate the plate at 37°C for 1 hr followed by 4°Covernight.*

*2. Wash the plate three times with washing buffer (PBS-T) (100µl with a multichannel pipette or by a wash bottle).*

*3.Prepare test serum, standard positive serum control and standard negative serum control separately in deep well plate at 1:100 dilution (2 µl serum + 198 µl BB) in 5% blocking buffer (BB).*

*3. Dispense 100µl of diluted standard positive serum (1:100 in duplicate wells) in **C1** and **D1;** standard negative serum in **E1** and **F1** and test sera to the respective wells from **2** to **12 (A to H)** according to the pre-planned test format and incubate the plate at 37°Cfor 1 h in water bath.*

*4. Wash the plate three times with washing buffer (PBS-T).*

*5. Add 50µl of diluted (1:2000 in blocking buffer) anti-pig IgG HRPO-conjugate to all the wells and incubate the plate at 37°C for 1 hr.*

*6. Wash the plate three times with washing buffer (PBS-T).*

*7. Add 50µl of freshly prepared chromogen-substrate mixture (OPD+$H_2O_2$) to all the wells and incubate the plate at 37°C for 10 to 15 minutes in the dark for colour development.*

*8. Add 50µl of stopping solution (1 M $H_2SO_4$) to all the wells and tap the plate gently to stop the color reaction.*

*9. Take reading at 490nm in an ELISA plate reader and determine the Optical density (OD) value of respective wells.*

### *Interpretation:*

*1. A test sample is considered positive when $OD_{490}$ of the sample is equals or more than 40% of the average $OD_{490}$ of the standard positive control.*

*2. Average $OD_{490}$ value of standard positive sample commonly ranges from 0.5 to 2.0*

*3. Average $OD_{490}$ value of standard negative sample commonly ranges from 0.04 to 0.15*


**ACKNOWLEDGEMENTS**

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and

Director of Research (Vety.) College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam.


| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | CC | T1 | | | | | | | | | | |
| B | CC | T1 | | | | | | | | | | |
| C | +VE | T2 | | | | | | | | | | |
| D | +VE | T2 | | | | | | | | | | |
| E | -VE | T3 | | | | | | | | | | |
| F | -VE | T3 | | | | | | | | | | |
| G | -Ag | T4 | | | | | | | | | | |
| H | -Ag | T4 | | | | | | | | | | |

TEST SAMPLES

| | |
|---|---|
| CC | Conjugate control (Blocking buffer + Substrate) |
| +VE | Known positive serum control (+VE Serum +Blocking buffer+Conjugate+Substrate) |
| -VE | Known negative serum control (-VE Serum +Blocking buffer+Conjugate+Substrate) |
| -Ag | Antigen blank(Blocking buffer+Conjugate +Substrate) without coated Antigen. |
| T1-T4 | Test samples (At the ratio 1:100) |