

RECOMBINANT ELISA FOR DETECTION OF ANTIBODIES AGAINST AFRICAN SWINE FEVER VIRUS

DESCRIPTION

Recombinant antigens for ELISA detection of ASFV p30-specific antibodies from pig serum samples. Boxes contain lyophilized positive antigen (Ag +) and negative antigen (Ag -) that should be rehydrated and used to coat ELISA plates. In order to determine the serological status of the individuals, each serum sample should be tested against both antigens. A list of the buffers and reagents needed for the assays is detailed at the end of this protocol.

METHODS

1. Rehydrate the Ag+ and ● Ag- vials, using carbonate-bicarbonate buffer 50mM, pH 9.6 (1 ml of per vial) and keep them on ice. These will be the stock antigens.
2. To coat the plates, dilute the stock antigens 1:20 in the same carbonate-bicarbonate buffer. For example; for 50 determinations use 250 µl of stock antigens and 4.75 ml of buffer. You can freeze the rest of the unused stock antigen at -20°C for further assays. Avoid more than 1 freezing-thawing cycle.
3. Coat ELISA plates using 100 µl of diluted recombinant antigen per well, following the diagram below. Each plate would be enough for 48 single determinations

	1	2	3	4	5	6	7	8	9	10	11	12
Ag+												
● Ag-												
Ag+												
● Ag-												
Ag+												
● Ag-												
Ag+												
● Ag-												

4. Incubate plates at 4°C overnight and the next day wash each well with ~300 µl PBST 3 times. Avoid plate drying between washing and prior to the addition of serum samples and conjugate. Following the final wash firmly tap residual buffer from the plates onto absorbent material.
5. Dispense 100 µl of PBST 2%BSA (blocking buffer) per well and incubate for 1 hour at 37°C After incubation, discard the blocking buffer and tap plates onto absorbent material without washing
6. Dilute serum samples 1:100 using blocking buffer and dispense 100 µl of the dilution in Ag+ and ●Ag- coated wells. Incubated for 1 hour at 37°C and repeat washing step as described in 4. It is recommendable to include positive and negative serum samples as controls for the assay.
7. Dilute anti-pig IgG-HRP conjugate 1:2000 in blocking buffer and dispense 100 µl/well. Incubate for 1 hour at 37°C and repeat washing step as described in 4 but washing 5 times instead of 3.

NOTE: it is possible to use protein A-HRP conjugate instead of the anti-pig IgG-HRP conjugate, the using dilution should be determined for each case.

8. Add 100 µl of substrate solution (ABTS-H₂O₂) per well and incubate protected from light at room temperature for approximately 5 minutes or until solid colour can be visualized in the positive control wells while negative control serum remains uncoloured. Plates may be directly read without stopping the reaction or colour development may be stopped using 1% SDS solution.

NOTE: it is possible to use other substrates as OPD-H₂O₂, once again the experimental conditions may vary from one substrate to other and should be determined previously.

9. Read plates at 405 nm wavelength and record absorbances obtained.

INTERPRETATION OF RESULTS

The colour intensity will be proportional to the amount of specific antibodies binding the antigen. ELISA results should be evaluated in relation to the ●Ag- by calculating the ratio for each sera: $R = \text{O.D Ag+} / \text{O.D } \bullet\text{Ag-}$

For R values lower than 2, the sample is classified as negative for antibodies to the p30 antigen of ASFV.

For R is greater than or equal to 2 , the sample is classified as positive for antibodies to the p30 antigen of ASFV.

BUFFERS REQUIRED

➤ **Carbonate/bicarbonate buffer 0.05M (pH 9.6)**

Na ₂ CO ₃ (Merck ref.1.06392)	1.59 g
NaHCO ₃ (Merck ref. 6329)	3.88 g
Distilled water to	1000 ml

Store at 4°C.

➤ **Washing solution: PBS 1x pH 7.2 - 0.1% Tween-20 (PBST)**

NaCl (Merck ref. 1.06404)	8.0 g
KH ₂ PO ₄ (Merck ref. 1.04873)	0.2 g
Na ₂ HPO ₄ 12 H ₂ O (Merck ref. 1.06586)	2.9 g
KCl (Merck ref. 1.04936)	0.2 g
Tween-20 (Merck ref. 8.22184)	1 ml
Distilled water to	1000 ml

Adjust pH before adding Tween 20 and store at + 4°C.

➤ **Blocking solution:**

2% (w/v) bovine serum albumin (BSA, Sigma ref. A-7906) in PBST

➤ **Substrate: ABTS**

ABTS peroxidase substrate (1 component) (KPL ref. 50-66-01)