UNIVERSIDAD COMPLUTENSE DE MADRID
Facultad de Veterinaria
Departamento de Sanidad Animal

REFERENCE LABORATORY

AFRICAN SWINE FEVER ANTIBODY DETECTION:

IMMUNOBLOTTING

ASSAY (IB)

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1. MATERIALS

Watch video (Immunoblotting)

- Single channel pipettes 1-10 µl
- Single channel pipettes 10-100 µl
- Single channel pipettes 10-200 µl
- Single channel pipettes 200-1000 µl
- Chamber 37ºC
- Analytical Balance
- Distilled water
- Minincubation trays (ref:170-3902.BIORAD)
- pH meter
- Tubes shaker or vortex mixer
- Reagent reservoir Polystyrene 50ml (ref: 4870.COSTAR)
- Sterile plastic tubes (10ml,50ml)
- Table centrifuge
- Latex or nitrile gloves

2. REAGENTS:

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➢ **Blocking solution: 2% (w/v) milk in PBS/Tween-20 pH 7,2**

PBS Buffer pH 7.2/ 0.05%Tween-20:

ClNa [Merck 1.06404]---------- 8 gr
ClK [Merck 1.04873]---------- 0,2 gr
PO_4H_2K [Merck 1.06586] ---------- 0,2 gr
PO_4HNa_2 [Merck 1.04936] ---------- 1,15gr
Tween-20 [Merck 8.22184] ---------- 0,5 ml
H_2O destilada ---------- 1000 ml

Check the pH before use. Store at 4ºC

PBS/ Tween 20, 2% milk Buffer :

Non fat dry milk (NESTLÉ-Sveltesse o Molico)----- 2g
Buffer PBS pH 7.2 – Tween-20 0.05%----------1000ml

Store at 4ºC. Do not use it after two days.

- **Conjugate:**

  Protein A peroxidase 1mg/ml (PIERCE, ref.0032400) store at 4ºC. Resuspend it in 200 µl of distilled water and store it at -20ºC. Before the adittion to immunoblotting strips, dilute it 1/1000 in PBS 1x/0,05% Tween-20 /2% Milk.

- **Substrate solution**:

  a) Disolve 6mg of 4-chloronaphtol in 2 ml of Metanol
  b) Add slowly 4-chloronaphtol/Metanol solution to 10 ml of PBS buffer pH 7.2 with vigorous agitation.
  c) Then, add 4 µl of H_2O_2 30% (Panreac) to the PBS/4-chloronaphtol solution.

3. METHODOLOGY

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3.1) Number the nitrocellulose strips.

3.2) Number the trays

3.3) Place the strips in the trays.

3.4) Block the nitrocellulose strips with blocking solution (1ml/strip) (PBS Buffer 7.2-Tween 20 0,05%-2% Milk)

3.5) Incubate for 30 minutes at 37ºC in continuous agitation.

3.6) Wash four times with PBS buffer pH 7.2-Tween 20 0.05%-2% Milk,1ml per strip.

3.7) Add 1ml of the test and control serum at a 1/40 dilution in blocking solution (PBS Buffer 7.2-Tween 20 0,05%-2% Milk).

3.8) Incubate it for 45 minutes at 37ºC in continuous agitation.

3.9) Wash four times with PBS buffer pH 7.2-Tween 20 0.05%-2% Milk,1ml per strip.

3.10) Preparation and addition of the conjugate:

11µl Protein A + 10.989 11µl PBS Buffer pH 7.2-Tween-20 0,05%, 2%milk (for un tray)

3.11) Incubate it for 45 minutes at 37ºC in continuous agitation.

3.12) Wash four times with PBS buffer pH 7.2-Tween 20 0.05%-2% Milk.

3.13) Substrate preparation:

6mg 4-chloronaphtol + 2ml Metanol + 10 ml PBS Buffer + 4 µl H_2O_2

3.14) 1ml of substrate is added

3.15) Stop the reaction after 10-15 minutes with distilled water.

4. READING AND INTERPRETING THE RESULTS

Sera showing a specific pattern of reaction similar as the antigen strips stained with positive control serum will be considered as positive to the ASF antibodies.

Any ELISA-positive or doubt serum that does not clearly react with the ASF proteins will be considered negative to ASF antibodies.