



OBTENTION OF THE AFRICAN SWINE FEVER (ASF) SOLUBLE CYTOPLASMIC ANTIGEN

• INTRODUCTION

A highly specific cytoplasmic soluble fraction of African swine fever (ASF) infected cells, is employed as the test antigen in the diagnosis of the ASF virus by ELISA, IEOP and Immunoblotting. This antigen is composed of all the ASF infection proteins, showing high sensitivity and it does not produce false positive reactions (Pastor et al., 1988; Escribano et al., 1989; Pastor et al., 1990).

• MATERIALS AND REAGENTS REQUIRED

TRIS HCl 1M pH=8

SUCROSE 0,34 M (w/v) in Tris HCl 5mM pH=8

SUCROSE 0,067 M (w/v) in Tris HCl 5mM pH=8

10% NP40 al 10% in distilled water

SUCROSE 64% (w/v) Tris HCl 0,4M.

EDTA 0,2 M.

TNE buffer

- 4,75 ml distilled water
- 1,4 ml EDTA 0,2 M
- 0,35 ml Tris HCl 1M pH 8
- 0,5 ml β-mercaptoetanol

CINa 5M

SUCROSE 20% (w/w) en Tris HCl 50 mM pH 8



• METHODS

The ELISA antigen is prepared from infected cells grown in the presence of pig serum.

- i) Infect MS (monkey stable) cells at multiplicity of infection of 10 with adapted virus, and incubate for two hours at 37 ° in continuous agitation (adsorption of the virus). After two hours complete with medium containing 2% pig serum.
- ii) Harvest the cells at 24-48 hours post-infection, when the CPE is extensive, and centrifuge at 650 **g** for 5 minutes
- iii) Wash the cell pellet in 0.34 M sucrose in 5 mM Tris-HCl, pH 8.0, and centrifuge at 1000 **g** for 5 minutes to pellet cells.
- iv) Remove the supernatant and resuspend the cell pellet in 67 mM sucrose in 5 mM Tris-HCl, pH 8.0 (1.8 ml per 175 cm² flask), and leave for 10 minutes on ice with agitation after 5 minutes.
- v) Add nonionic detergent Nonidet P-40 to a final concentration of 1% (w/v), and leave for 10 minutes (with agitation after 5 minutes) to lyse the cells.
- vi) Add sucrose to a final concentration of 64% (w/w) in 0.4 M Tris-HCl, pH 8.0, and centrifuge at 1000 **g** for 10 minutes to pellet nuclei.
- vii) Collect the supernatant and add EDTA (2 mM final concentration), beta-mercaptoethanol (50 mM final concentration) and NaCl (0.5 M final concentration) in 0.25 mM Tris-HCl, pH 8.0, and incubate for 15 minutes at 25°C. In this step the viral particles are disaggregated and the NaCl maintain the isotonicity.
- viii) Centrifuge at 100,000 **g** for 1 hour at 4°C over a layer of 20% (w/w) sucrose in 50 mM Tris-HCl, pH 8.0.
- ix) Remove the band immediately above the sucrose layer and use as the ELISA antigen or IB antigen. Store at (20°C).

REFERENCES

Pastor, M.J. Sánchez-Vizcaíno, J.M., Escribano, J.M. (1988). Dos nuevas técnicas para el diagnóstico de la PPA: Immunoelctro-transferencia y Enzimo-inmuno-adsorción. *Med. Vet.* 5 (5-6). 275-282.

Escribano, J.M., Pastor, M.J. y Sánchez-Vizcaíno, J.M. (1989). Antibodies to bovine serum albumin in swine sera: Implications for false-positive reactions in the serodiagnosis of African swine fever. *Am. J. Vet. Res.* 50 (7), 1118-1122.

Pastor, M.J., Arias, M. y Escribano, J.M. (1990). Comparison of two antigens for use in an enzyme-linked immunosorbent assay to detect African swine fever antibody. *Am. J. Vet. Res.* 51(10), 1540-1543

ASF reviews:

- Arias, M., Sánchez-Vizcaíno, JM (2002). African Swine Fever (ASF). In *Trends in Emerging Viral Infections of Swine*. Iowa State University press, ISBN: 0813803837. Eds. A. Morilla, K-J Yoon, J. Zimmerman. Pp 119-124.
- Arias, M., Sánchez-Vizcaíno, JM (2002). African Swine Fever Eradication: The Spanish model. In *Trends in Emerging Viral Infections of Swine*. Iowa State University press, ISBN: 0813803837. Eds. A. Morilla, K-J Yoon, J. Zimmerman. pp 133-139.
- Arias, M; Sánchez, C; González, MA; Carrasco, L; y Sánchez-Vizcaíno, JM. (2002). "Peste porcina Africana" In *curso digital de enfermedades infecciosas porcinas*. www.sanidadanimal.info on line, July, 2002.

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