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Anti-Classical Swine Fever Virus Monoclonal Antibody

BIO 275

Reagent for indirect immunofluorescence or peroxidase

REAGENT FOR DETECTION OF CSFV ON TISSUE SECTION OR CELL CULTURE

INTRODUCTION

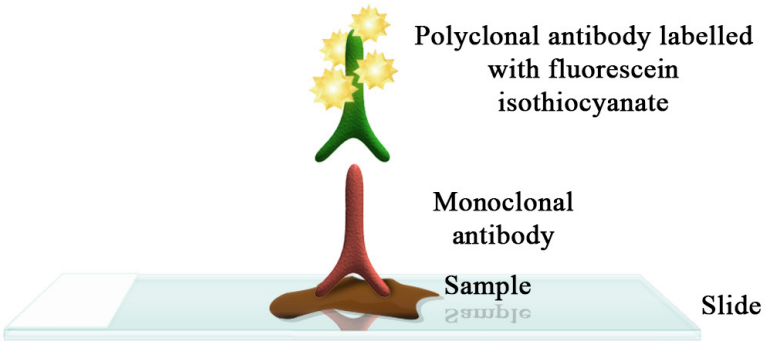
Classical swine fever (CSF) or hog cholera is an extremely contagious disease of swine with high mortality, especially in young pigs. It is caused by a small 44 nm-in-diameter enveloped virus belonging to the genus Pestivirus (Flaviviridae family). The CSF virus is transmitted by direct or indirect contact between animals via the blood, tissues, secretions, and excretions of sick or dead animals. The routes of infection are ingestion, inhalation, genital infection, and skin abrasions.

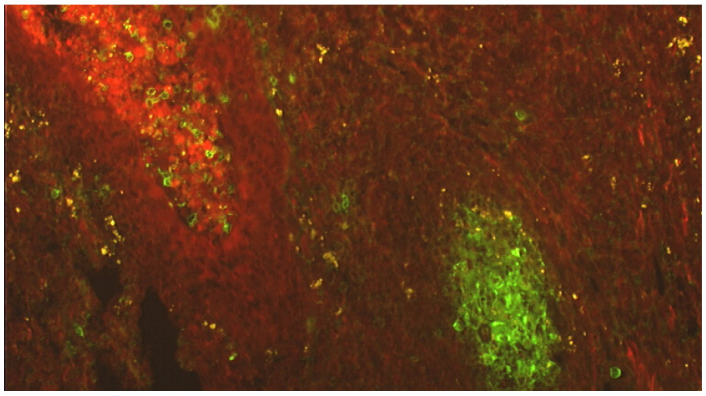
The disease is present across large areas of Asia, Central America, and South America, as well as in some parts of Europe and Africa. Many countries are free of the disease. The official diagnosis of classical swine fever currently recognised by the International Office of Epizootics (OIE) entails isolating the viral strains in a susceptible cell line and identifying them by an immunological test (direct or indirect immunofluorescence assay or direct or indirect immunoperoxidase assay). ELISA tests are also recognised, but only as serological tests.

BIO 275 reacted with all CSFV strains tested (21 strains originated from Belgium, France, Germany, Switzerland, Austria, The Netherlands, USA, Italy and Czech republic. BIO 275 did not show any reactivity with 9 BVDV strains tested.

BIO 275 is specific for E2 protein.

EXAMPLE OF RESULTS





Bio-X Diagnostics - 38, Rue de la Calestienne (PAE) - 5580 Rochefort - Belgique
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I – INDIRECT IMMUNOFLUORESCENCE ASSAY PROCEDURE

Fix the cell preparation (cell culture or tissue sections) for 15 minutes at room temperature using one of the fixatives listed below:

- 2% paraformaldehyde in PBS
- 9:1 (v/v) acetone/water solution
- Pure isopropanol solution
- Absolute ethanol solution

Then rinse with PBS.

Dilute the reagent twentyfold in a PBS-Evans Blue solution prepared according to the following formula:

PBS-Evans Blue

NaCl:	8 gm
KH2PO4:	0.2 gm
KCl:	0.2 gm
Na2HPO4 . 2H2O:	1.15 gm
Evans Blue:	0.01 gm
NaN3:	0.1 gm
H20	1 L

Incubate the preparation on the sample for 1 hour at room temperature, preferably in a humidity chamber.
Upon completion of this incubation period rinse the preparation with a PBS solution.
Then add the conjugate (fluorescein-labelled anti-mouse immunoglobulin) at the manufacturer's recommended dilution. The conjugate available from Bio-X Diagnostics (Bio 305) should be diluted twentyfold in PBS-Evans Blue solution.
Incubate the preparation on the sample for 1 hour at room temperature, preferably, in a humidity chamber.
After this second incubation step rinse the preparation with PBS.

Dry the slide, then add the mounting medium made up as follows:

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Mounting medium

Glycerol	9 parts by volume
PBS	1 part by volume

Place a cover slip on the slide, then observe under a microscope fitted for fluorescence detection.

The antibody may be kept in its original vial at 4°C for more than a year. Never freeze this reagent. Once diluted in the PBS-Evans Blue solution, the antibody remains stable for one week at 4°C.

II – INDIRECT IMMUNOPEROXIDASE ASSAY PROCEDURE

Fix the cell preparation (cell culture or tissue sections) for 15 minutes at room temperature using one of the following fixatives:

- 2% paraformaldehyde in PBS
- 9:1 (v/v) acetone/water solution
- Pure isopropanol solution
- Absolute ethanol solution

Then rinse with PBS.

Dilute the reagent twentyfold in PBS prepared according to the following formula:

PBS

NaCl:	8 gm
KH2PO4:	0.2 gm
KCl:	0.2 gm
Na2HPO4 . 2H2O:	1.15 gm
NaN3:	0.1 gm
H20	1 L

Incubate the preparation on the sample for 1 hour at room temperature, preferably in a humidity chamber.
Upon completion of this incubation period rinse the preparation with PBS.
Then add the conjugate (peroxidase-coupled anti-mouse immunoglobulin) at the manufacturer's recommended dilution. The conjugate available from Bio-X Diagnostics (Bio 269) should be diluted fiftyfold in PBS.
Incubate the preparation on the sample for 1 hour at room temperature, preferably in a humidity chamber.
After this second incubation step rinse the preparation with PBS.
Then add the chromogen (AEC, precipitating TMB, DAB, etc.) and the substrate (hydrogen peroxide) according to the manufacturer's instructions. Examine under the microscope for the presence of the coloured marker.

COMPOSITION: One vial of 500 µl

STORING THE REAGENT: The antibody must be stored at 4°C. It must never be frozen.

STABILITY: One year at 4°C

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