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ANTI-CLASSICAL SWINE FEVER VIRUS MONOCLONAL ANTIBODY (BIO 275)

(Reagent for indirect immunofluorescence or immunoperoxidase assay)

REAGENT FOR DETECTING CSFV ON CELL CULTURES.

I – INDIRECT IMMUNOFLUORESCENCE ASSAY PROCEDURE

Fix the cell culture 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 buffer

PBS

NaCl:	8 gm
KH_2PO_4:	0.2 gm
KCl:	0.2 gm
$Na_2HPO_4 . 2H_2O$:	1.15 gm
NaN_3:	0.1 gm
H_20	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.

PBS-Evans Blue

NaCl:	8 gm
KH_2PO_4:	0.2 gm
KCl:	0.2 gm
$Na_2HPO_4 . 2H_2O$:	1.15 gm
Evans Blue:	0.01 gm
NaN_3:	0.1 gm
H_20	1 L

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:

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 PBS, the antibody remains stable for one week at 4°C.

II – INDIRECT IMMUNOPEROXIDASE ASSAY PROCEDURE

Fix the cell culture 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
KH_2PO_4:	0.2 gm
KCl:	0.2 gm
$Na_2HPO_4 . 2H_2O$:	1.15 gm
NaN_3:	0.1 gm
H_20	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 twentyfold 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.