Anti-Bovine PARAINFLUENZA 3 Monoclonal Antibody

BIO 290

Reagent for indirect immunofluorescence or peroxidase

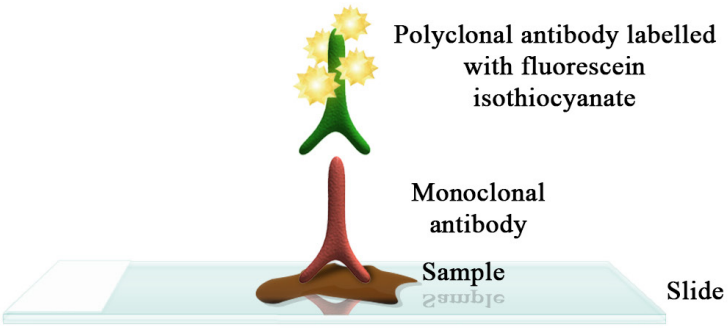
REAGENT FOR DETECTION OF BOVINE PARAINFLUENZA 3 ON TISSUE SECTION

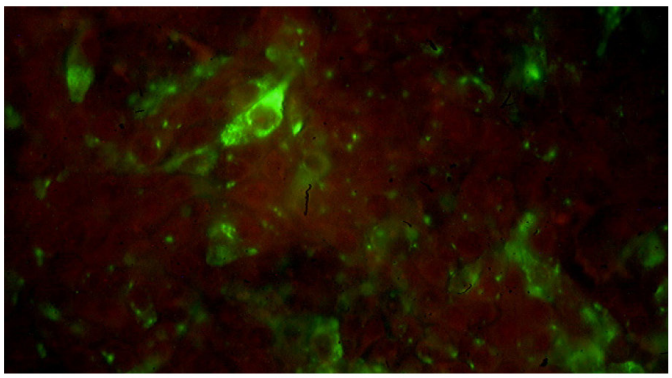
OR CELL CULTURE

INTRODUCTION

Parainfluenza 3 was first isolated in the USA from the nasal mucus of cattle showing clinical signs of shipping fever. Its distribution in cattle has been found to be worldwide. Most reports of bovine PI3 virus activity have been in groups of young cattle with respiratory diseases such as enzootic calf pneumonia and shipping fever. Bovine PI3 virus infections are not invariably associated with disease, and subclinical infections often occur. In Europe, PI3 infection mostly occurs during the months from October to March. PI3 virus infection may be accompanied by concurrent infection of the respiratory tract by other viruses such as respiratory syncytial virus, adenovirus, and BVDV. In outbreaks of bovine respiratory disease, it is not possible to diagnose PI3 virus infection on clinical grounds alone. The direct immunofluorescence assay enables one to detect the presence of PI3 in frozen tissue sections made from lung fragments (preferable from the cranioventral lobes at the boundary between the diseased and apparently normal tissue) or epithelial tissue from the upper respiratory tract (large bronchi, trachea, and pituitary mucosa). The reagent can also be used to identify the virus's presence on an infected cell culture.

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:

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.

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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 CONJUGATE: The conjugate must be stored at 4°C. It must never be frozen.

STABILITY: One year at 4°C

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