Anti-Bovine Virus Diarrhoea Virus Monoclonal Antibody

BIO 295

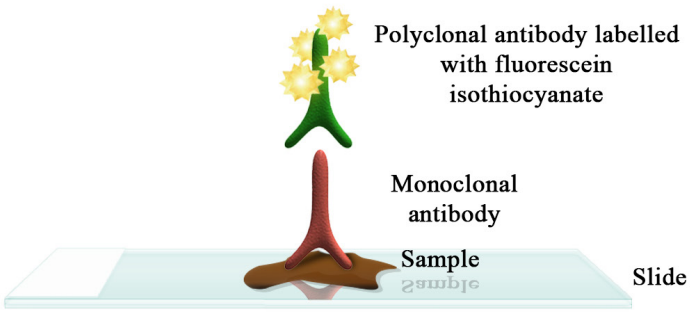
Reagent for indirect immunofluorescence or immunoperoxidase

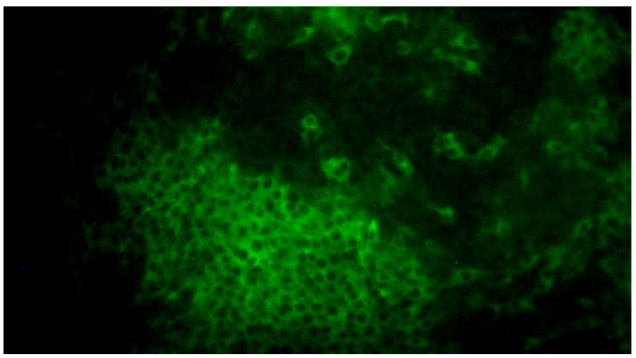
REAGENT FOR DETECTION OF BVDV ON TISSUE SECTION OR CELL CULTURE

INTRODUCTION

BVD--bovine virus diarrhoea--and mucosal disease (MD) complex are two different clinical disorders caused by the same virus. BVD is the result of an acute infection in susceptible animals. Onset may occur at any time after birth. BVD has a brief course and low mortality. Mucosal disease, in contrast, is a deadly disease of low morbidity. It develops in viraemic animals that have been contaminated in utero. The characteristic of this in utero infection is the existence of specific immunotolerance that prevents the animals from producing antibody against the infective strain but not against another, antigenically different, BVD strain. These persistent carriers, which can live for years without developing clinical signs of the disease, can be detected by laboratory screening tests only. The direct immunofluorescence assay allows one to detect the presence of BVDV on frozen tissue sections made from fragments of respiratory organs (lungs), digestive organs (buccal or oesophageal mucosa, intestines), or lymphoid tissue (lymph nodes, spleen, Peyer's patches). The reagent can also be used to identify the virus's presence on an infected cell culture.

EXAMPLE OF RESULTS





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

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.

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

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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