Anti-IHNV Monoclonal Antibody

BIO 285

Reagent for indirect immunofluorescence or peroxidase

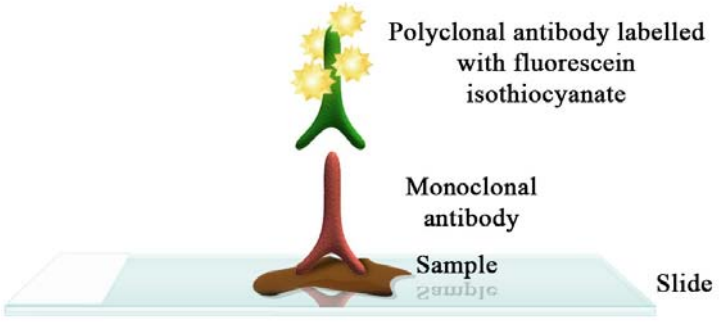
REAGENT FOR DETECTION OF INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

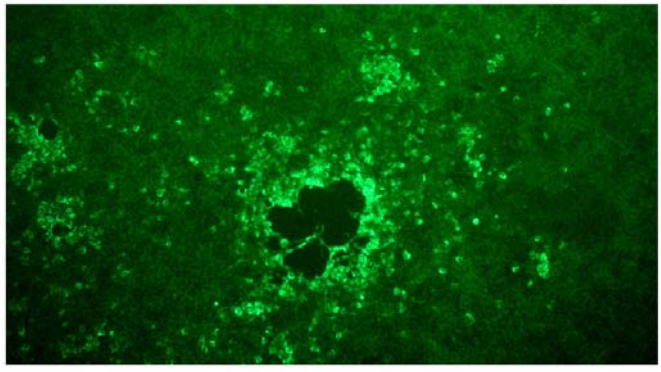
ON TISSUE SECTION OR CELL CULTURE (136/3)

INTRODUCTION

Infectious haematopoietic necrosis (IHN) is confined mainly to the North American Pacific Coast, certain countries of the Far East and some European countries. This viral infection is caused by a rhabdovirus. There are currently 4 or 5 known subgroups of this virus. Several Salmonidae species are sensitive to the virus. They include various Pacific salmon species (*Onchorhynchus sp.*), the Atlantic salmon (*Salmo salar*) and the rainbow trout (*Onchorhynchus mikiss*). The brown trout (*Salmo trutta fario*) has recently been shown to be sensitive to the virus. Pike fry (*Esox lucius*) is sensitive to the virus experimentally. The clinical disease generally occurs in water at temperature below 14° C. It is characterized by nervous system and digestive disorders: alternating apathy and spasmodic movements and enteritis as evidenced by long, whitish excrement. Autopsy reveals exophtalmia, ascites and haemorrhages in the muscle mass and viscera . The mortality rates associated with the virus can be high.
It is practically impossible to distinguish VHS from infectious haematopoietic necrosis (IHN) - another Salmonidae viral infection likewise caused by a rhabdovirus - on the basis of clinical examination alone. A differential diagnosis must thus be performed in a laboratory. The clinical diagnosis is most often confirmed by isolating the virus in a cell culture.
BIO 285 is specific for IHNV N-protein.

EXAMPLE OF RESULTS





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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

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
- Pure isopropanol 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

Dilutions	Strains/Virus	Cells	Results
1:20	IHN 265 (Isolate 233)	RTG-2	+
1:20	None	RTG-2	-
1:20	IHN 265 (Isolate 233)	EPC	+
1:20	None	EPC	-
1:20	IHN 265 (Isolate 332 from Germany)	RTG-2	+
1:20	IHN 260 (Isolate K3)	RTG-2	+
1:20	IHN 259 (Isolate DF04/99 from Germany)	RTG-2	+
1:20	IHN 269 (N61)	RTG-2	+
1:20	IHN 249 (Strain 4008 from Italy)	RTG-2	+
1:20	IHN 265 (Isolate 332 from Germany)	EPC	+
1:20	IHN 260 (Isolate K3)	EPC	+
1:20	IHN 259 (Isolate DF04/99 from Germany)	EPC	+
1:20	IHN 269 (N61)	EPC	+
1:20	IHN 249 (Strain 4008 from Italy)	EPC	+

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