Anti-VHSV Monoclonal Antibody

BIO 282

Reagent for indirect immunofluorescence or peroxidase

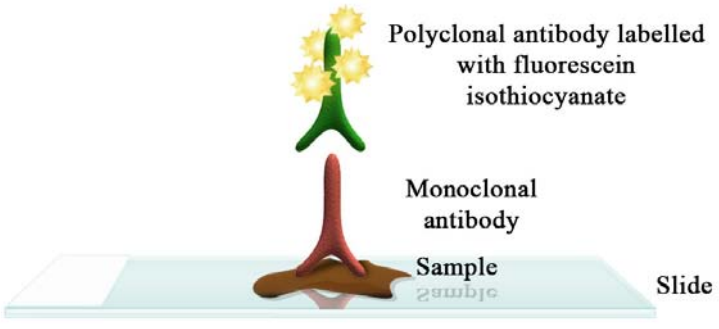
REAGENT FOR DETECTION OF VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

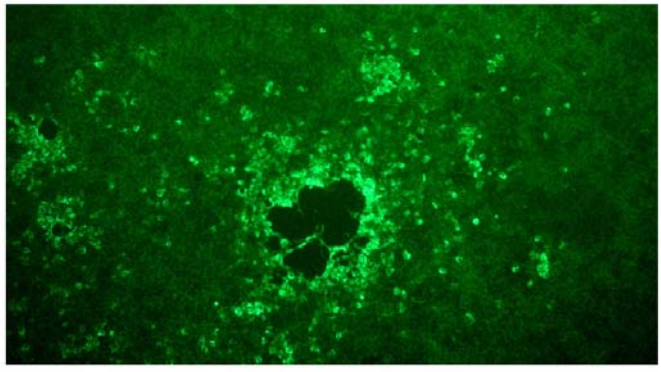
ON TISSUE SECTION OR CELL CULTURE (IP5B11)

INTRODUCTION

Viral haemorrhagic septicaemia (VHS) is a rhabdovirus infection that is very common on the European continent. It causes great losses on European trout farms - an estimated 20 to 30 thousand tons of trout are lost to this disease each year. Three distinct serotypes of this virus have been identified. The rainbow trout (*Oncorhynchus mykiss*) appears to be very sensitive to this virus. However, other Salmonidae, such as the brown trout (*Salmo trutta fario L.*), grayling (*Thymallus thymallus L.*) and even white fish may develop the disease. Salmonidae hybrids show variable degrees of sensitivity to the virus. The pike (*Esox lucius L.*) is also sensitive to VHS, primarily as fry, but the adult fish is also vulnerable. The pathogenic power of this virus is expressed only in water that is colder than 14° C. This explains why the disease strikes primarily in the winter.
The clinical signs of the disease are high mortality, especially during the young trout's first winter. The subjects exhibit melanosis and exophthalmia. The paleness of their gills reflects their anaemic condition. An autopsy will reveal the presence of numerous sites of haemorrhages in the viscera and muscle mass. 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 282 is specific for VHSV 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	VHS 903 (Fi13)	RTG-2	+
1:20	None	RTG-2	-
1:20	VHS 903 (Fi13)	EPC	+
1:20	None	EPC	-
1:20	VHS 903 (Fi13)	RTG-2	+
1:20	VHS 922 DF72/94 (isolate Wi)	RTG-2	+
1:20	VHS 917 DK-3592 «Voldbjerg»	RTG-2	+
1:20	VHS 958 07/71 (France)	RTG-2	+
1:20	VHS 919 1P8 (Isolate from herring)	RTG-2	+
1:20	VHS 903 (Fi13)	EPC	+
1:20	VHS 922 DF72/94 (isolate Wi)	EPC	+
1:20	VHS 917 DK-3592 «Voldbjerg»	EPC	+
1:20	VHS 958 07/71 (France)	EPC	+
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