



Anti-ISA Monoclonal Antibody

BIO 337

Reagent for indirect immunofluorescence or peroxidase

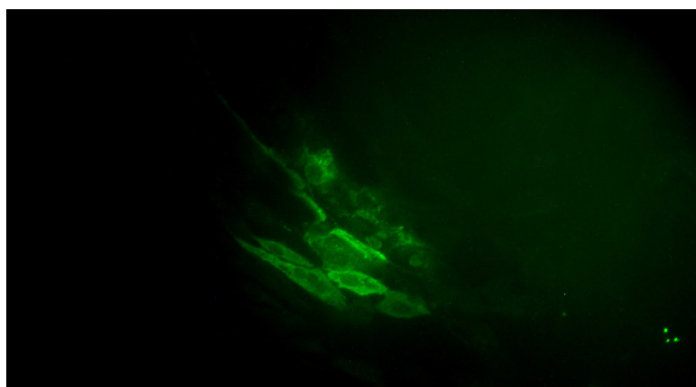
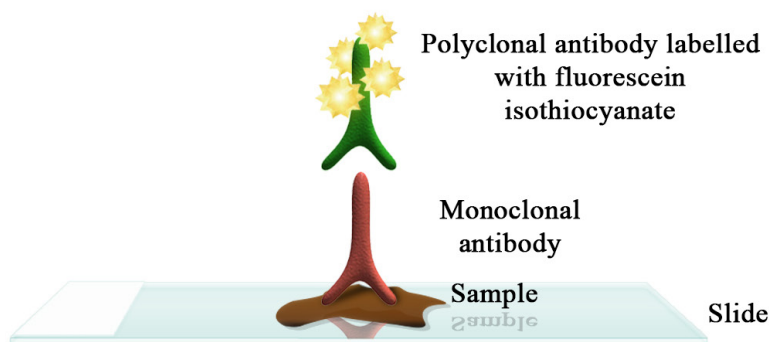
REAGENT FOR DETECTION OF INFECTIOUS SALMON ANAEMIA VIRUS

ON TISSUE SECTION OR CELL CULTURE

INTRODUCTION

Infectious salmon anaemia (ISA) is a contagious infectious disease caused by an Orthomyxoviridae. It affects the Atlantic salmon (*Salmo salar* L.), causing high mortality in this species (15 to 100% mortality in affected tanks). ISA has been diagnosed in Norway since 1984, in Canada since 1997, and in Scotland since 1998. The disease is characterised by severe anaemia (hematocrit < 10), lymphocytopenia, and thrombocytopenia. ISA can be diagnosed by immunofluorescence assays on cryosections of specific tissues, isolating the virus on cell cultures (SHK-1 cells) with immunofluorescence confirmation, and PCR. The target organs for an immunofluorescence assay are the kidney, heart, and liver. BIO 337 is specific for N protein

EXAMPLE OF RESULTS





I – INDIRECT IMMUNOFLUORESCENCE ASSAY PROCEDURE

Fix the cell culture for 15 minutes using one of the fixatives listed below:

- 9:1 (v/v) acetone/water solution at room temperature
- 5:5 (v/v) acetone/ethanol at - 20°C

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
KH ₂ PO ₄ :	0.2 gm
KCl:	0.2 gm
Na ₂ HPO ₄ . 2H ₂ O:	1.15 gm
Evans Blue:	0.01 gm
NaN ₃ :	0.1 gm
H ₂ O	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 culture for 15 minutes using one of the fixatives listed below:

- 9:1 (v/v) acetone/water solution at room temperature
- 5:5 (v/v) acetone/ethanol at - 20°C

Then rinse with PBS.

Dilute the reagent twentyfold in PBS prepared according to the following formula:

PBS

NaCl:	8 gm
KH ₂ PO ₄ :	0.2 gm
KCl:	0.2 gm
Na ₂ HPO ₄ . 2H ₂ O:	1.15 gm
NaN ₃ :	0.1 gm
H ₂ O	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
1:8	Glesvaer 2/90 (Norway)	SHK-1	+++
1:16	Glesvaer 2/90 (Norway)	SHK-1	+++
1:32	Glesvaer 2/90 (Norway)	SHK-1	++
1:64	Glesvaer 2/90 (Norway)	SHK-1	+
1:128	Glesvaer 2/90 (Norway)	SHK-1	0
1:20	390/98 (Scotland)	SHK-1	+++
1:20	ILA 31 (Canada, 1997)	SHK-1	+++
1:20	ILA 35 (Canada, 1998)	SHK-1	+++
1:20	ILA 65 (Canada, 2001)	SHK-1	+++
orthomyxo-virus	DF 29/03-1671 (Koï)	CCB	-
VHSV	F1, Fi 13	RTG-2	-
VHSV	Klapmoelle (DK)	RTG-2	-
VHSV	23.75 (F)	RTG-2	-
SVCV	RC 56/70	FHM	-
SVCV	DF 17/00	FHM	-
IHN	Isolat 233 (D)	EPC	-
IHN	32/87 (F)	EPC	-
IPNV	LWVRT 60-1 (USA)	EPC	-
IPNV	Abild (DK)	EPC	-

