



# Anti-SVCoV Monoclonal Antibody

BIO 331

Reagent for indirect immunofluorescence or peroxidase

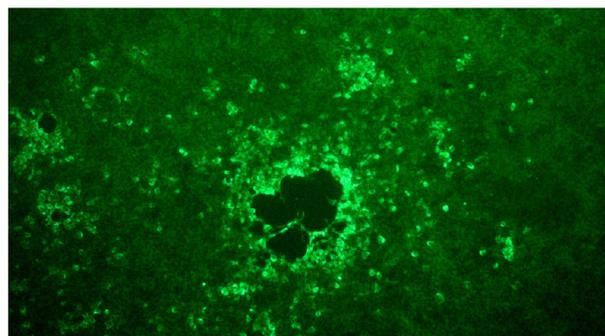
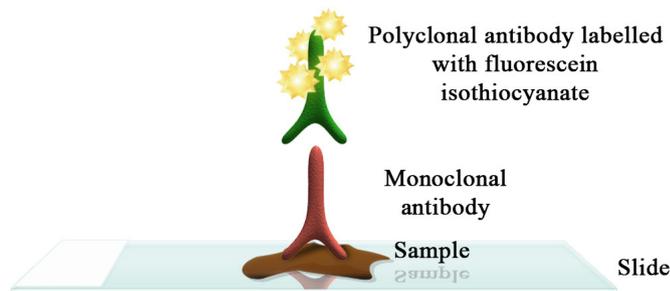
REAGENT FOR DETECTION OF SPRING VIRAEMIA OF CARP VIRUS

ON TISSUE SECTION OR CELL CULTURE

## INTRODUCTION

Spring viraemia of carp is a contagious viral disease of the Cyprinidae. Other species, such as the sheatfish (*Silurus glanis*), are also sensitive to this virus. The cause of the disease is a rhabdovirus named Rhabdovirus carpio. This viral infection is found in all parts of Europe where Cyprinidae are farmed, but the disease also exists in other areas. It affects fish of all ages, the most typical victims being the «one summer» fry at the time of the spring warming of the water. The disease has a high death rate. The clinical signs of contamination are petechial haemorrhages of the skin and gills, dark coloring of the tegument, exophthalmia and a distended abdomen. Loss of balance is also seen in diseased fish. The internal lesions are characterised by petechial haemorrhages of the viscera, fibrinous peritonitis, and catarrhal or necrotic enteritis. Whilst the serological traces of an SVC viral infection indicate that serology may be a valid alternative for studying the health status of a carp population, laboratory diagnosis of the disease usually involves identification of the virus in cell cultures.

## 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
- 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

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NaCl:	8 gm
KH <sub>2</sub> PO <sub>4</sub> :	0.2 gm
KCl:	0.2 gm
Na <sub>2</sub> HPO <sub>4</sub> . 2H <sub>2</sub> O:	1.15 gm
Evans Blue:	0.01 gm
NaN <sub>3</sub> :	0.1 gm
H <sub>2</sub> 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 156) 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

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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 between +2°C and +8°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 between +2°C and +8°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

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NaCl:	8 gm
KH <sub>2</sub> PO <sub>4</sub> :	0.2 gm
KCl:	0.2 gm
Na <sub>2</sub> HPO <sub>4</sub> . 2H <sub>2</sub> O:	1.15 gm
NaN <sub>3</sub> :	0.1 gm
H <sub>2</sub> 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 157) 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 between +2°C and +8°C. It must never be frozen.

STABILITY: One year between +2°C and +8°C





Dilutions	Strains/Virus	Cells	Results
1:10	RVB-405 SVCV RC 56/70		++
1:20	RVB-405 SVCV RC 56/70		++
1:10	RVB-423 SVCV DF 01/01 V1592		+++
1:20	RVB-423 SVCV DF 01/01 V1592		++
1:10	RVB-424 SVCV DF 01/01 V1621		+++
1:20	RVB-424 SVCV DF 01/01 V1621		+++
1:10	RVB-425 SVCV DF 19/00 52/94		+++
1:20	RVB-425 SVCV DF 19/00 52/94		++
1:10	RVB-426 SVCV DF 19/00 59/95		+++
1:20	RVB-426 SVCV DF 19/00 59/95		+++
1:10	RVB-427 SVCV DF 19/00 73/94		+++
1:20	RVB-427 SVCV DF 19/00 73/94		+++
1:10	RVB-428 SVCV DF 17/00 47/3		+++
1:20	RVB-428 SVCV DF 17/00 47/3		+++
1:10	RVB-429 SVCV Stendal 1230		+++
1:20	RVB-429 SVCV Stendal 1230		++
1:10	RVB-430 SVCV Stendal 1231		+++
1:20	RVB-430 SVCV Stendal 1231		+++
1:10	SVCV 1237 RC 56/70		+
1:20	SVCV 1237 RC 56/70		+
1:10	DF 10/01-VF366 884 1732		+
1:20	DF 10/01-VF366 884 1732		+
1:10	DF 10/01-VF366 885		+
1:20	DF 10/01-VF366 885		+
1:10	RVB-X127 VHSV DF 72/94 (Type Wi)		0
1:20	RVB-X127 VHSV DF 72/94 (Type Wi)		0
1:10	RVB-X132 IHNV DF 4/99-8/99-E3		0
1:20	RVB-X132 IHNV DF 4/99-8/99-E3		0
1:10	RVB-X201 Eel Rhabdovirus DF 59/96-121-1		0
1:20	RVB-X201 Eel Rhabdovirus DF 59/96-121-1		0
1:10	RVB-431 PFRV PFR 42		0
1:20	RVB-431 PFRV PFR 42		0
1:10	PFRV PFR 43		+
1:20	PFRV PFR 43		(+)
1:10	PFRV PFR 44		+
1:20	PFRV PFR 44		+

