



IHNV ELISA KIT

BIO K 273/1 - BIO K 273/2

Infectious haematopoietic necrosis (IHN) is a viral disease caused by a rhabdovirus. It affects most salmonid species, especially the fry and young fish. Susceptible species include rainbow or steelhead trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*); Pacific salmon, including Chinook (*O. tshawytscha*), sockeye (*O. nerka*), chum (*O. keta*), masou (*O. masou*) and coho (*O. kisutch*); and Atlantic salmon (*Salmo salar*). The disease causes sometimes extremely high economic losses, whether in fresh water populations or seawater fisheries. The clinical disease generally occurs in water at temperatures between +8°C and +15°C. It is characterized by nervous system and digestive disorders: alternating apathy and spasmodic movements, darkening of the skin, pale gills and a distended abdomen. Enteritis is evidenced by long, whitish excrement. Autopsy reveals exophthalmos, ascites and haemorrhages in the muscle mass and viscera. The liver, kidney and spleen are pale. The mortality rates associated with the virus can be high. It is almost impossible to distinguish IHN from viral haemorrhagic septicaemia (VHS), another Salmonidae viral infection likewise caused by a rhabdovirus, on the basis of clinical evidence alone. A differential diagnosis obtained by laboratory investigation thus appears to be indispensable.

EIA Procedure

- 1- Microplate coated with monoclonal antibodies
- 2- Add samples and positive control.
Incubate 1 hour at 21°C +/- 3°C
Wash
- 3- Add conjugate.
Incubate 1 hour at 21°C +/- 3°C .
Wash
- 4- Add chromogen (TMB)
Wait 10 minutes.
Add stop solution.
Read at 450 nm



Reliable Results

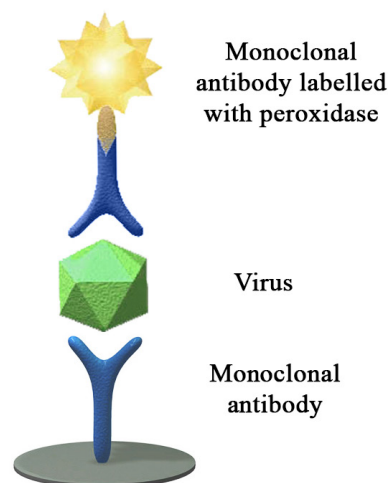
The use of monoclonal antibodies as conjugate and capture ensure excellent specificity and very reliable results.

Ease-of-Use

Minimal hands-on-time
Room temperature incubation
Results available in 140 minutes.

Flexibility

Results can be read visually or spectrophotometrically.





Example of results

Nationales Referenzlabor für Fischkrankheiten,
im Institut für Infektionsmedizin
Insel Riems, 19.09.06

Cell culture

RTG-2, CCLV, Rainbow trout gonade

FHM, CCLV, Feed head minnow

EPC, CCLV, Epithelioma papulosum cyprini

Viruses Names	Strains
VHS1014	strain TUV, Denmark
VHS1015	Riemser VHS-Vakzine
VHS1016	reference strain Klapmølle (Denmark)
VHS1017	reference strain 23.75 (France)
VHS1018	Ö62/96, Österreich
VHS1019	Strain 07/71, France
VHS1020	reference strain Voldbjerg (Denmark)
VHS1022	Laborstamm Fi13 (ENZMANN)
VHS1034	Laborstamm Fi13 (ENZMANN)
VHS1036	Isolat 05/00, Deutschland
VHS1037	marine isolate IP8 (herring)
VHS1038	Isolat DF72/94 (Germany)
VHS-Pool 1039/40/41	Laborstamm Fi13 (ENZMANN),TV-Infektionsvirus
SVC1231	Isolat VF, Deutschland
SVC1232	Isolat
SVC1233	Isolat DF 17/00, Deutschland
SVC1234	Isolat DF 17/00 (Germany)
SVC1238	reference strain RC 56/70 (FIJAN)
IHN252	Isolate 4008, Italy
IHN259	Isolat Df 04/99, Deutschland
IHN260	Isolat KINKELIN; France
IHN274	Isolate 4008, Italy
IHN280	isolate 233 (Germany)
IPN449	reference strain Abild, Ab (Denmark)
IPN450	reference strain Spjarup, Sp (Denmark)
IPN451	Birnavirus II, CRL Aarhus, Denmark
IPN452	reference strain Abild, Ab (Denmark)
IPN453	reference strain VR299 (U.S.A.)
IPN455	reference strain VR299 (U.S.A.)
IPN457	reference strain Abild (Denmark)
IPN459	reference strain Spjarup, Sp (Denmark IPN455)



Sensitivity IHNV Batch: IHN06D24 Datum 22.06.06

Virus Titre	IHN252 (NT/ml)	IHN259 (4.5/ml)	IHN260 (4.0/ml)	IHN274 (6.5/ml)	IHN280 (6.5/ml)
Dilution					
10 ⁰	0.979 +	2.445+	2.195+	2.212+	2.355+
10 ¹	0.166+	2.065+	0.627+	1.167+	1.487+
10 ²	0.105-	0.646+	0.165+	0.115-	0.238+
10 ³	-0.003-	0.069-	-0.018-	0.004-	-0.067-
10 ⁴	0.003-	0.030-	-0.124-	0.030-	0.012-
10 ⁵	-0.006-	-0.076-	0.026-	-0.033-	0.025-
Titre	5.5/ml	2.5/ml	2.0/ml	5.5/ml	4.5/ml
Positive control from the kit: 2.136					

Specificity IHNV Batch: IHN06D24 Datum 03.07.06

Strains (pool)	Dilution	OD	Status
VHS1022	undiluted	0	negative
VHS1019	undiluted	-0.003	negative
VHS1018	undiluted	-0.003	negative
VHS1017	undiluted	-0.001	negative
VHS1016	undiluted	0.001	negative
VHS1038	undiluted	0.006	negative
VHS1037	undiluted	-0.002	negative
VHS1036	undiluted	-0.004	negative
VHS1015	undiluted	0.004	negative
VHS1014	undiluted	0.001	negative
SVC1238	undiluted	-0.003	negative
SVC1234	undiluted	0.002	negative
SVC1232	undiluted	0.002	negative
SVC1231	undiluted	0	negative
IHN280	undiluted	2.732	Positive
Positive control		2.719	Valid





Conclusions

IHNV detectability between $10^{2,0}$ and $10^{5,5}$ / ml

No crossreactivity detected with other Rhabdoviruses.

The ELISA tests from Bio-X Diagnostics allow detection of IHNV after cell culture amplification.

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ISOLATE	Geno- type	Anti-VHSV anti- body		Anti-IHNV antibody	
		Delta OD	Val	Delta OD	Val
IHNV					
HAG		0,003	0	1,853	82
RBH		0,002	0	1,535	68
TR		0,004	0	2,006	89
FR-32/87		0,045	2	2,052	91
I-4008		0,012	1	,577	25
Colemann		0,005	0	1,569	69
OSV		0,004	0	1,634	72
ER		0,003	0	1,115	49
AU-9695338		0,000	0	2,005	89
DF 13/98-11621		0,002	0	1,546	68
US-DW		-0,005	0	2,044	90
Control BioX		1,961	100	2,265	100





Conclusions

The Bio-X IHNV ELISA kit detects all the tested IHNV isolates with correct results.
The specificity is 100 % by testing all VHSV positive in the IHNV test.

Composition of the kit

BIO-X IHNV ELISA KIT : BIO K 273

	BIO K 273/1	BIO K 273/2
Microplates	1 (48 tests)	2 (96 tests)
Washing solution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Conjugate	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Positive control	1 X 2 ml (1 X)	1 X 4 ml (1 X)
Single component TMB	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Stopping solution	1 X 6 ml (1 X)	1 X 12 ml (1 X)

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

