



DUO VHSV - IHNV ELISA KIT

BIO K 264/2

Viral haemorrhagic septicaemia (VHS) is a disease of farmed rainbow trout, farmed turbot, farmed Japanese flounder as well as several wild freshwater and marine species caused by VHSV rhabdovirus. Disease generally occurs at temperature between +4°C and +14°C. At water temperature between +15°C and +18°C, the disease generally takes a short course with a modest accumulated mortality. Disease rarely occurs at higher temperatures. VHS outbreaks occur during all seasons, but are most common in spring when water temperatures are rising or fluctuating. The clinical signs of the disease are high mortality (which can reach up to 100% in fry), especially during the young trout's first winter. The subjects exhibit lethargy, 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, distended abdomen due to oedema in the peritoneal cavity. VHS can also occur in a nervous form, characterised by severe abnormal swimming behaviour, such as constant flashing and/or spiralling. 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*), 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 clinical disease generally occurs in water at temperature between +8 and +15°C. It is characterized by nervous system and digestive disorders: alternating apathy and spasmodic movements, darkening of the skin, pale gills and distended abdomen. Enteritis is evidenced by long, whitish excrement. Autopsy reveals exophthalmia, 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 VHS on the basis of clinical evidence alone. A differential diagnosis obtained by laboratory investigation thus appears to be indispensable. The VHS-IHN ELISA test confirms the virus's growth on a susceptible cell line.

EIA Procedure

- 1- Microplate coated with monoclonal or polyclonal antibodies
- 2- Add samples and positive controls.
Incubate 1 hour at 21°C +/- 3°C
Wash
- 3- Add conjugates.
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 antibody as conjugate ensures excellent specificity and very reliable results.

Ease-of-Use

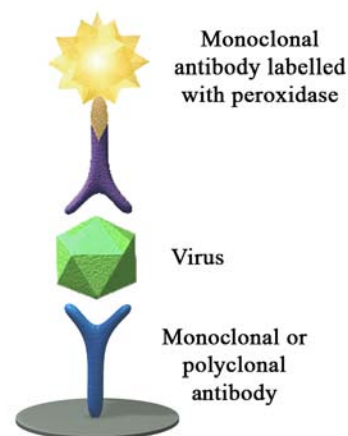
Minimal hands-on-time
Room temperature incubation
Results available in 140 minutes.

Flexibility

Results can be read visually or spectrophotometrically.



Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it





Example of results

Cell culture

RTG-2, CCLV, Rainbow trout gonade

FHM, CCLV, Feed head minnow

EPC, CCLV, Epitheilioma 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 (FIJIAN)
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 VHSV Batch: VHS06D24 Datum 21.06.06

Virus Titre	VHS1017 (6.5/ml)	VHS1018 (6.0/ml)	VHS1019 (7.5/ml)	VHS1020 (5.5/ml)	VHS1036 (NT/ml)	VHS1037 (5.5/ml)	VHS1038 (6.5/ml)	VHS-pool 1039/40/41 (7.75/ml)
Dilution								
10^0	0.686 +	0.332+	1.240+	0.253+	1.399+	0.223+	0.914+	1.801+
10^1	0.165+	0.127-	1.240+	0.121-	0.249+	0.077-	0.228+	0.470+
10^2	0.084-	0.065-	0.077-	0.084-	0.077-	0.077-	0.068-	0.08-
10^3	0.103-	0.071-	0.087-	0.029-	0.083-	0.059-	0.117-	0.046-
10^4	0.073-	0.087-	0.001-	0.069-	0.095-	0.043-	0.061-	0.051-
10^5		0.122-	0.058-	0.07-	0.027-	0.049-	-0.037-	0.071-
Titre	5.5/ml	6.0/ml	6.5/ml	5.5/ml	?/ml	5.5/ml	5.5/ml	6.75/ml
Positive control from the kit: 2.180								

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

Specificity VHSV Batch: VHS06D24 Datum 04.07.06

Strains (pool)	Dilution	OD	Status
IHN280	undiluted	0.006	negative
IHN274	undiluted	0.146	negative
IHN260	undiluted	0.108	negative
IHN259	undiluted	0.080	negative
IHN252	undiluted	0.033	negative
SVC1238	undiluted	0.065	negative
SVC1233	undiluted	0.067	negative
SVC1232	undiluted	0.068	negative
SVC1231	undiluted	0.088	negative
VHS1016	undiluted	0.774	positive
VHS1015	undiluted	0.413	positive
VHS1014	undiluted	1.055	positive
VHS1034	undiluted	0.695	positive
Positive control		2.292	Valid



Conclusions

VHSV detectability between $10^{5,5}$ and $10^{6,75}$ / ml

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

No crossreactivity detected with other Rhabdoviruses.

The European Community Reference Laboratory for Fish Diseases

National Veterinary Institute

Technical University of Denmark

Hangovej 2

DK-8200 Aarhus N

Denmark

December 18, 2009

ISOLATE	Geno- type	Anti-VHSV anti- body		Anti-IHNV antibody	
		Delta OD	Val	Delta OD	Val
DK-F1	I	1,293	72	-0,001	0
DK-Hededam	I	0,395	22	0,011	1
DK-3592B	Ia	0,696	39	0,041	2
DK-3971	Ia	0,961	53	-0,003	0
DK-3946	Ia	0,984	55	0,001	0
DK-5151	Ia	0,589	33	-0,002	0
DK-6137	Ia	0,789	44	-0,011	-1
DK-7974	Ia	1,059	59	0,006	0
DK-9695377	Ia	1,069	59	0,001	0
DK-200051	Ia	1,024	57	-0,002	0
DK-200149	Ia	1,198	67	0,002	0
FR-07-71	Ia	0,916	51	0,000	0
FR-23-75	Ia	1,097	61	-0,011	-1
FR-02-84	Ia	0,455	25	0,002	0
CZ-R5	Ia	0,761	42	-0,017	-1
CZ-2077	Ia	1,088	60	-0,008	0
DK-5927	Ia	1,104	61	-0,001	0
AU-8/95	Ia	0,742	41	0,001	0
CH-F1 262 BFH	Ia	0,467	26	-0,004	0
PL-202473	Ia	1,101	61	-0,006	0



ISOLATE	Geno- type	Anti-VHSV anti- body		Anti-IHNV antibody	
		Delta OD	Val	Delta OD	Val
DK-M Rhabdo	Ib	0,963	54	-0,005	0
1p8	Ib	0,850	47	-0,004	0
Ap40	Ib	0,814	45	-0,006	0
Control BioX		1,800	100	2,101	100
1p85	Ib	0,867	41	0,004	0
1p86	Ib	0,779	37	0,000	0
1p93	Ib	1,007	48	0,002	0
1p116	Ib	0,971	46	-0,001	0
1p20	Ib	0,896	42	0,002	0
1p121	Ib	0,998	47	-0,004	0
5p276	Ib	0,530	25	-0,004	0
SE-SVA-14	Ib	0,851	40	0,001	0
SE-SVA-1033	Ib	1,139	54	-0,002	0
UK-96-43	Ib	0,808	38	-0,005	0
4p37	Ib	0,285	13	0,000	0
FiA01a00 200198-1	Id	0,788	37	-0,002	0
FiP02b00 200240	Id	0,520	25	0,031	1
No-A136-68 EG46	Id	0,651	31	-0,024	-1
Ge-1.2	Ie	0,395	19	-0,003	0
TR-206239-1	Ie	0,890	42	0,020	1
1p49	II	0,990	47	-0,001	0
1p52	II	0,387	18	0,022	1
1p53	II	0,780	37	0,000	0
1p54	II	0,800	38	-0,002	0
2p51	III	0,950	45	-0,002	0
4p101	III	0,867	41	0,005	0
4p168	III	0,845	40	-0,013	-1
Control BioX		2,113	100	2,292	100
DK-4p51	III	1,019	49	0,001	0
UK-H17/5/93	III	0,954	46	-0,004	0
UK-860/94	III	1,389	66	-0,001	0
UK-H17/2/95	III	1,027	49	-0,005	0



ISOLATE	Geno- type	Anti-VHSV anti- body		Anti-IHNV antibody	
		Delta OD	Val	Delta OD	Val
F-L59x	III	0,983	47	0,000	0
GH30	III	0,569	27	-0,002	0
IR-F13.02.97	III	1,063	51	0,000	0
NO-2007-50-385	III	0,994	47	-0,001	0
USA-Makah	IVa	0,624	30	0,003	0
USA-KHV	IVa	0,672	32	-0,003	0
USA-Elliot Bay	IVa	0,626	30	0,000	0
Minter Creek, WA	IVa	0,903	43	-0,005	0
Tokul Creek, WA	IVa	0,988	47	-0,002	0
Port Angels, WA	IVa	0,849	41	-0,001	0
BC93	IVa	0,624	30	-0,001	0
CAN-3624	IVa	0,821	39	0,000	0
CAN-99-019	IVa	0,826	39	0,003	0
Quatsini, BC	IVa	0,919	44	-0,003	0
JP-Obama 25	IVa	0,570	27	-0,004	0
JF00Ehi1	IVa	0,557	27	-0,003	0
BRO1Ehi1	IVa	0,309	15	0,001	0
JF01Oit1	IVa	0,241	12	0,000	0
JSL02 Yaml	IVa	0,740	35	-0,005	0
Control BioX		2,095	100	2,395	100
PMO5Ehi1	IVa	0,496	25	-0,007	0
Lake St. Clair	IVb	0,715	36	0,002	0
Goby 1-5	IVb	0,775	40	-0,007	0
Lake Ontario, NY	IVb	0,693	35	0,003	0
Budd Lake, MI	IVb	0,805	41	-0,001	0
Skaneateles Lake, MI	IVb	0,820	42	-0,005	0
New Brunswick	IVb	0,940	48	0,002	0
1p40	Ib	0,848	43	-0,002	0
DK-5131	Ia	0,633	32	-0,004	0
DK-2835		0,976	50	-0,002	0
DK-5123		1,056	54	-0,004	0
Skaneateles Lake, 2 clone	IVb	0,712	36	0,004	0
Control BioX		1,961	100	2,265	100



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 VHSV-IHNV ELISA kit detects all the tested VHSV and IHNV isolates with correct results.

The specificity is 100 % by testing all IHNV or VHSV positive in the VHSV-IHNV test.



Composition of the kit

BIO-X DUO VHSV - IHNV ELISA KIT : BIO K 264

	BIO K 264/2
Microplate	2 (48 tests)
Washing solution	1 X 100 ml (20 X)
Conjugates	2 X 12 ml (1 X)
Control antigen	1 X 4 ml (1 X)
Single component TMB	1 X 25 ml (1 X)
Stopping solution	1 X 15 ml (1 X)

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

