Primerdesign

R01075

Infectious Salmon Anaemia Virus

Kit version: 1

Target region:

ISAV-NA HPR0 (Haemagglutinin-esterase (HE) gene)

ISAV-EU HPR0 (Haemagglutinin-esterase (HE) gene)

ISAV (M1 gene)

genesig®PLEX Kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

Infectious salmon anaemia virus (ISAV) is classified into two major genetic groups: the European (EU) and the North American (NA) clades. The ISAV RNA genome comprises eight single-stranded, negative-sense RNA segments that encode at least 10 proteins including haemagglutinin esterase (HE). Within the HE gene is a highly polymorphic region (HPR) that is a determinant of virulence. Deletions in this polymorphic region (HPRΔ) can cause disease (virulent), whereas the genotype with no deletion (HPR0) is not associated with disease (avirulent). Both the EU and NA clades have HPR0 and HPRΔ genotypes.

The genesig®PLEX ISAV kit is designed for the in vitro detection of virulent ISAV and avirulent ISAV in both the EU and NA clades.

Specificity

The kit is designed for the in vitro detection of ISAV genomes and to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**ISAV primer/probe mix (100 reactions)** FAM labelled, Target: ISAV-NA HPR0 Cy5 labelled, Target: ISAV-EU HPR0 ROX labelled, Target: ISAV (EU & NA) VIC labelled, Target: Internal extraction control		BROWN
1	**ISAV positive control template**		RED
1	**Internal extraction control RNA (150 reactions)**		BLUE
2	**oasig®PLUS OneStep Lyophilised qPCR Master Mix** **(50 reactions per glass vial)**		GOLD
2	**oasig® resuspension buffer** For resuspension of the oasig®PLUS OneStep Lyophilised qPCR Master Mix		BLUE
1	**RNase/DNase free water** for resuspension of primer/probe mixes		WHITE
1	**Template preparation buffer** for resuspension of internal control template and positive control template		YELLOW

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes

qPCR plates or reaction tubes

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and RNA integrity. An internal extraction control is provided to test for non-specific PCR inhibitors.

Dynamic range of test

Under optimal PCR conditions the assays can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

A target specific primer and probe mix is provided, and amplification can be detected through the FAM, Cy5, ROX and VIC channels.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5′-dye and a 3′-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

The kit contains a positive control. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the targets worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false- positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Internal RNA extraction control

When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

Within the multiplex primer/probe mix are primers and probes to detect the exogenous RNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control RNA does not interfere with detection of the target RNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/-3 depending on the level of sample dilution.

Resuspension protocol

To minimize the risk of contamination with foreign RNA/DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mixes in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
ISAV primer/probe mix (**BROWN**)	110 µl

3. **Resuspend the oasig®PLUS OneStep Lyophilised qPCR Master Mix in oasig® resuspension buffer, according to the table below:**

Component - resuspend in oasig® resuspension buffer	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)	525 µl

4. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control RNA (**BLUE**)	600 µl
Post-PCR heat-sealed foil	
ISAV Positive Control Template (**RED**) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4 µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer.**

2. **Complete RNA extraction according to the manufacturer's protocols.**

OneStep RT-qPCR detection protocol

For optimum performance and sensitivity.
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each RNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (GOLD)	10 µl
ISAV primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15 µl of these mixes into each well according to your experimental plate set up.**

3. **Pipette 5 µl of RNA sample into each well according to your experimental plate set up.**
   For negative control wells use 5 µl of RNase/DNase free water (WHITE). The final volume in each well is 20 µl.

4. **Pipette 5 µl of positive control template into each well according to your experimental plate set up.**
   The positive control template (RED) contains templates for ISAV (EU and NA), ISAV-NA HPR0 and ISAV-EU HPR0. The final volume in each well is 20 µl.

OneStep RT-qPCR Amplification Protocol

Recommended amplification conditions when using oasig®PLUS OneStep Lyophilised qPCR Master Mix.

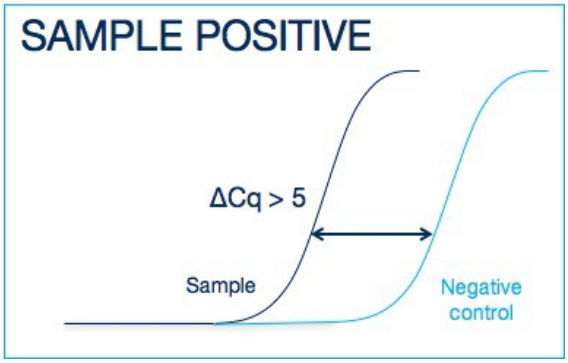
	Step	Time	Temp
	Reverse Transcription	10 min	55 ℃
	Enzyme activation	2 min	95 ℃
Cycling x50	Denaturation	10 s	95 ℃
	DATA COLLECTION *	60 s	60 ℃

* Fluorogenic data should be collected during this step through the FAM, Cy5, ROX and VIC channels.

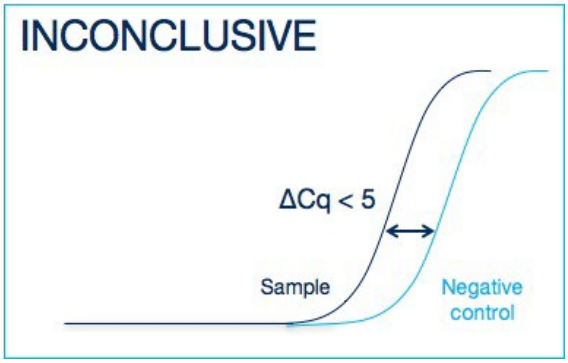
Interpretation of results

Target (FAM)	Target (Cy5)	Target (ROX)	Internal extraction control (VIC)	Positive control	Negative control	Interpretation
-	-	+	+ / -	+	-	**ISAV virulent (EU or NA) POSITIVE RESULT**
-	+	+	+ / -	+	-	**ISAV-EU avirulent POSITIVE RESULT**
+	-	+	+ / -	+	-	**ISAV-NA avirulent POSITIVE RESULT**
-	-	-	+	+	-	**NEGATIVE RESULT**
+ / -	+ / -	+ / -	+ / -	+	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	+ / -	+ / -	+ / -	+	> 35	*
-	-	-	-	+	-	**SAMPLE PREPARATION FAILED**
+ / -	+ / -	+ / -	+ / -	-	+ / -	**EXPERIMENT FAILED**

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Positive Control

The positive control is expected to amplify between Cq 16 – 23 in the FAM, Cy5 and ROX channels. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised and should be repeated.

Internal PCR control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

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