Spring viraemia of carp virus - Real Time RNA

50 / 100 /150 tests (Ready to use kit)

SVCV RNA Real Time PCR Kit is a screening assay for rapid and accurate detection of Spring viraemia of carp virus.

Test principles

This One Step qRT-PCR Kits provides compo-nents for One Step reverse transcription and quantitati-ve PCR (qRT-PCR) in a convenient format that is compa-tible with both rapid and standard qPCR cycling condi-tions. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. These One Step qRT-PCR kits have been formulated for use with fluorogenic probe-based 5' nuclease technology probes.
The One qPCR Enzyme Mix includes Reverse Transcrip-tase, Recombinant Ribonuclease Inhibitor in an optimi-zed formulation, Taq DNA polymerase, and all reagents for an optimized qRT-PCR.
Specific primer and probe mix for Spring viraemia of carp virus are provided in the kit and these can be detec-ted through your Real Time thermal cycler by the 5' nuclease PCR detection method. During PCR amplifica-tion, forward and reverse primers hybridize to the Spring viraemia of carp virus target cDNA. A fluorogenic probe is included in the same reaction mixture, which consists of a DNA probe labeled with a 5-reporter FAM and 3-quencher which can be detected through the green channel **(see Table 8).**
To confirm the extraction of a valid biological template, an Internal control primer and probe mix are included, which consist of a DNA probe labeled with a 5-reporter HEX and a 3-quencher, which hybridize to a specific housekeeping endogenous target gene. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated.
As a result, a fluorescence increase can be detected on a range of real time PCR platforms through the yellow channel **(see Table 8).**
Our RNA Kits include an exogenous source of RNA tem-plate. A separate qPCR primer/probe mix is supplied with this kit to detect an exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentra-tions, which consist of a DNA probe labeled with a 5-dye FAM and a 3-quencher that, during PCR amplification, a fluorescence increase can be detected through the green channel of Real Time thermal cyclers. Successful co-purification and qPCR for the control RNA indicates that PCR inhibitors are not present at a high concentra-tion **(see Table 8).**
Our kits also include Positive and Negative Controls which are detailed in the FAQ section.

Principle of use

This amplification kit has been manufactured to detect Spring viraemia of carp virus via Real Time PCR. This is a relative/absolute quantification or qualitative assay.

Real Time PCR is based on fluorogenic dyes. Ct values between 12 – 36 should be taken as positive. Values between 36-40 Ct should be taken as marginal positive. Ct values above 40 must be considered as negative **(for more details see Table 5).**

This kit needs RNA as a template which can be isolated from blood, serum, faeces, respiratory fluid, cerebrospi-nal fluid, digestive system, tissues, egg yolk, milk, swabs, bacterial cultures, cell lines, among others.

Table 1. Kit Components

Reactions Tubes	50 test	100 test	150 test
Universal qPCR Master Mix	(1 unit)	(1 unit)	(1 unit)
Primer, Probes and Internal Control Mix	(1 unit)	(1 unit)	(1 unit)
One qRTPCR Enzyme Mix	(1 unit)	(1 unit)	(1 unit)
SVCV Positive Control	(1 unit)	(1 unit)	(1 unit)
SVCV Negative Control	(1 unit)	(1 unit)	(1 unit)
PCR grade Water	(1 unit)	(1 unit)	(1 unit)
Exogenous RNA control	(1 unit)	(1 unit)	(1 unit)
Exogenous RNA control Primer and Probes Mix	(1 unit)	(1 unit)	(1 unit)

Table 2. Instrument Compatibility

* ABI 7300	* LightCycler 480
* ABI 7500FAST	* Mastercycler® ep realplex
* ABI 7900	* Mx3000P QPCR System
* AB Step One	* Mx3005P QPCR System
* AB Step One Plus	* RotorGene 3000
* Agilent Mx3005P	* RotorGene 6000
* CFX96 & CFX384	* RotorGeneQ
* ExiCyclerTM 96	* QuantGene 9600
* iQ5 & MyiQ Cycler	* QuantStudio™ 5
* Illumina Eco	* SLAN® Real-Time PCR
* LightCycler Nano	* Smartcycles II
* LightCycler 2.0	* Applied 7300 and 7500

Procedure

Please read through the entire procedure before star-ting.

Before Starting

- Pulse-spin each tube in a centrifuge before opening.
- Homogenize the solution for 5 seconds prior to pipetting.
- Use different tips in order to avoid cross contamination.
- Use sterile, RNAse, DNAase, and pyrogen-free tips.
- Add Exogenous RNA Control to the lysis/binding solution concentrate that is used for the RNA isolation. Add 2 µL of undiluted Exogenous RNA Control (20,000 copies) per desirable isolation.

Step 1

Prepare a Master mix according to the Table 3.

Table 3. Reaction components for PCR

Reaction Tubes	Sample and Internal Control	Positive Control	Negative Control	Exogenous RNA Control
Universal qPCR Master Mix	7,5 µL	7,5 µL	7,5 µL	7,5 µL
Primer, Probes and Internal Control Mix	0,3 µL	0,3 µL	0,3 µL	0,3 µL
One qRTPCR Enzyme Mix	0,3µL	0,3µL	0,3µL	0,3µL
Exogenous RNA control Primer and Probes Mix				3,5 µL
PCR grade Water	3,4 µL	3,4 µL	3,4 µL	3,4 µL
RNA Sample	3.5 µL			
Positive Control		3.5 µL		
Negative Control			3.5 µL	
Total Volume	15 µL	15 µL	15 µL	15 µL

Step 2

Place the tubes in a thermal cycler and perform One Step qPCR according to the program outlined in Table 4.

Table 4. PCR Cycling table

Cycles	Steps	Time	Temp. (°C)
1 Cycle	Activation Step	2 min	25 °C
1 Cycle	Reverse Transcription	15 min	50 °C
1 Cycle	Denaturation	2 min	95 °C
40 Cycles	Annealing	30 seg	95 °C
	Extension	30 seg	55 °C
	Hold	-	4 °C

* Hold Step is optional.
* Measure the fluorescence at the end of the Extension Step.

Interpretation of the test

1) Qualitative analysis:

Ct (Threshold cycle) value of each sample can be read as follows.

Table 5. Ct value result

Ct value	Result
0 - 11	**Negative**
12 - 36	Positive
36 - 40	Marginal Positive
> 40	**Negative**

* Ct values over 40 are considered a Negative result. If the Ct value is in the 12 - 36 range, it is considered as Positive. This depends of the sample initial concentration used for each reaction. Note that the sample real concentration could be modified by the sample purity when it is quantified.

2) Quantitative analysis:

Table 6. Preparation of standard curve dilution series. SVCV positive control:

Average Positive Control Concentration	
SVCV	See Quality Control

Standard curve	Preparation series a fresh dilution	Concentration	Number of copies
Tube N°1:	2uL Positive Control (0,1 ng/µL) + 18 µL de PCR grade Water	See quality control	See quality control
Tube N°2:	2uL Tube N°1 + 18 µL de PCR grade Water	See quality control	See quality control
Tube N°3:	2uL Tube N°2 + 18 µL de PCR grade Water	See quality control	See quality control
Tube N°4:	2uL Tube N°3 + 18 µL de PCR grade Water	See quality control	See quality control
Tube N°5:	2uL Tube N°4 + 18 µL de PCR grade Water	See quality control	See quality control
Tube N°6:	2uL Tube N°5 + 18 µL de PCR grade Water	See quality control	See quality control
Tube N°7:	2uL Tube N°6 + 18 µL de PCR grade Water	See quality control	See quality control

Note: Don't forget to homogenize the tubes.

* We will send a Quality Control report for each purchase.
* For reaction mix you must use Universal qPCR Master Mix.
* If you want to obtain less DNA copies, include a new dilution tube (Tube N° 8). Final DNA copy number will depend of the DNA concentration (check the Quality Control Report).

Table 7. Standard curve set up

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F	Tube G
Universal qPCR Master Mix	7,5 µL	7,5 µL	7,5 µL	7,5 µL	7,5 µL	7,5 µL	7,5 µL
Primer, Probes and Internal Control Mix	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL
Enzyme Mix	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL
PCR Grade Water	3,4 µL	3,4 µL	3,4 µL	3,4 µL	3,4 µL	3,4 µL	3,4 µL
Tube N° 1 (Positive Control)	3,5 µL						
Tube N° 2		3,5 µL					
Tube N° 3			3,5 µL				
Tube N° 4				3,5 µL			
Tube N° 5					3,5 µL		
Tube N° 6						3,5 µL	
Tube N° 7							3,5 µL
Total Volume	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL

2.1.- Assess the Ct value when the amplification curve of Standard tubes 1, 2, 3, 4, 5, 6 passes the threshold line. Four tubes are sufficient for a standard curve (tubes 1 to 4).
2.2.- Calculate the quantitative value in order to compare the Ct value of the unknown samples with the standard curve.
2.3.- When visualizing the result for the Positive Control in the Real Time thermal cycler, there will be only one amplification curve for the target gene. It will not have an amplification curve for the Internal Control.

3) Test validation

3.1.- Each Ct value standard should be as follows.
Standard 1 < Standard 2 < Standard 3 < Standard 4 < Standard 5 < Standard 6.
3.2.- R-value of standard curve should be 0.900 - 0.999. R-value represent how well the experimental data fits the regression line. A significant difference in observed in Ct values between replicates will lower the R-value.
3.3.- The standard curve slope result should be negative.
3.4.- The desired amplification efficiencies vary from 90% to 110%. The theoretical maximum of 100% indicates that the polymerase enzyme is functioning at its maximum capacity. Low reaction efficiencies may be caused by poor primer design or by suboptimal reaction conditions. Reaction efficiencies >110 may indicate pipetting error in your serial dilutions or coamplification of nonspecific products, such as primer-dimers.

Visual explanation FAQ

1.-Positive control

The Positive control assay uses a FAM dye and should be detected through the green channel of your Real Time thermal cycler **(see Table 8)**.
For copy number determination and as a positive control for the PCR setup, the kit contains a plasmidial positive control template. This can be used to generate a standard curve of Spring viraemia of carp virus copy number / Ct value.

Alternatively, the positive control can be used at a single dilution if full quantitative analysis of the sample is not required. Each time the kit is used, at least one positive control reaction must be included in the run. **We recommend running a positive control for each 12 samples.** A positive result indicates that the primer and probes for the target gene worked properly in that particular experimental scenario. If a negative result is obtained the test results should be invalid and must be repeated **(see Table 10).** Seal all other samples and negative controls before pipetting the positive control into its well.

2.-Internal Control

The internal control is included in the Primer, Probes and Internal Control Mix alongside the target pathogen detection. The internal control assay uses a HEX dye and should be detected through the Yellow channel of your Real Time PCR instrument and should result in a Ct value of 28 (+/-5), depending on the level of sample dilution and concentration. Therefore, a positive result through the yellow channel indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the Yellow channel the results should be analyzed by combination of result, follow the **Table 10** data.

3.-Negative control

To confirm absence of contamination, a Negative control reaction should be included every time the kit is used. We recommend runing a Negative control for each 12 samples. In this instance, the PCR grade water should be used in place of template. A negative result indicates that the reagents are not contaminated.

If a positive result and a Ct value less than 36 is obtained, the results should be analyzed and checked if a correct amplification curve was obtained. If you obtain a clear amplification curve, you should consider repeating your assay due to sample contamination **(see Table 10)**.

* Remember: Run a positive control and negative control for each 12 samples. For reaction mix you must use Universal qPCR Master Mix.

4.-Internal RNA control

This control is an exogenous source of RNA template, of which its cDNA amplification of this control does not interfere with detection of the target cDNA, even at low copy numbers.

The Internal control is detected through the Green channel and gives a Ct value up to 30 depending on the level of sample dilution. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration. To confirm this, Internal RNA control should be included every time the kit is used.

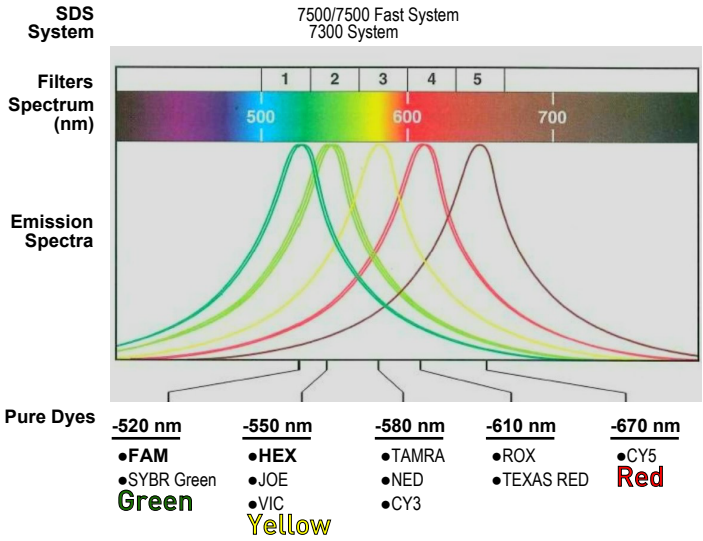
Particularly, due to amount of this reagent, we recommend including and running one Internal RNA control for each 12 samples.

* Exogenous RNA control is just referential and its negative result should not interpreted as a failed assay when the sample and internal control give a positive result (see Table 10).

* In order to setup your software to read the probes, see **Table 8** and run the following channels:

Table 8. Fluorogenic probes, Channels and Dyes

Channel	Source	Detector	Dyes
Green	470 nm	520 nm	**FAM**, SYBR Green I, Fluorescein, EvaGreen, Alexa Fluor 488.
Yellow	530 nm	550 nm	**HEX**, TET, CAL Fluor Gold 540, JOE, VIC, YaKima Yellow.
Orange	585 nm	610 nm	ROX, CAL Fluor Red 610, Cy3.5, Texas Red, Alexa Fluor 568.
Red	625 nm	660 nm	Cy5, Quasar 670, Lightcycler, Red 640, Alexa Fluor 633.
Crimson	680 nm	710 nm	Quasar 705, Lightcycler Red 705, Alexa Fluor 680.



Important Note:

Probes for the sample and controls mentioned in this manual are just referential and the purchased kits might have different ones. This information will be detailed in your Certificate of Analysis upon purchasing one of our PCR Kits.

We can develop special requests for other pathogens or multiplex detection according to the client specifications.

We strongly recommend to not use or combine our products with reagents from another kits or unknown procedence. We cannot assure good results if incompatibility problems occur.

Temperature

All our reagents are made through protein engineering and are stable at room temperature, the label temperature is just a recommendation after the product is open.

Table 9. Store Temperature Kits

Store temperature	The label temperature
Shipping temperature	At room temperature

Table 10. Interpretation of Results

Sample	I.C	N.C	P.C	eRNA Control	Result
+	+	-	+	+	POSITIVE
+	-	-	+	+	POSITIVE
+	+	-	+	-	POSITIVE
+	-	-	+	-	POSITIVE
+	+	+	+	+	Check Ct and Consider repeat assay*
+	-	+	+	+	Check Ct and Consider repeat assay*
+	+	+	+	-	Check Ct and Consider repeat assay*
+	+	-	-	-	Check Ct and Consider repeat assay*
+	+	+	-	-	Check Ct and Consider repeat assay*
+	+	-	-	+	Failed experiment
+	+	+	-	+	Failed experiment
+	-	-	-	-	Failed experiment
+	-	-	-	+	Failed experiment
+	-	+	-	-	Failed experiment
-	+ / -	+ / -	+ / -	+ / -	NEGATIVE

* Sometimes amplification curves for Negative or Internal control with Ct < 30 can be seen, however it might not be a Positive result. If the amplification curve is not sigmoid, you should consider it as a Negative result.

Table 11. Products Specifications

Technology	5' nuclease probe based real time PCR assay
Type of nucleic acid Kit	RNA
Kit storage	Shipped at room temperature, the label temperature is just a recommendation after the product is open.
Detection Limit	See Quality Control file. **Request it!**
Sensitivity & Specify	Ct values between 12 – 36 should be taken as positive. Values between 36-40 Ct should be taken as marginal positive. Ct values above 40 must be considered as negative.
Controls included	Internal control, Internal RNA extraction control, Positive control and Negative control
Channels	FAM Green channel detects pathogen amplicons. HEX Yellow channel detects internal control amplicons. FAM Green channel detects Positive Control. FAM Green channel detects Exogenous RNA control.

Gentaur Molecular Products BVBA
Address: Voortstraat 49, 1910 Kampenhout, Belgium
T: 0032 16 58 90 45 | **E:** info@gentaur.com
Websites: www.gentaur.com | www.maxanim.com