

PCRmax Ltd™ qPCR test

# Slow Bee Paralysis virus

Polyprotein gene

150 tests

For general laboratory and research use only



# Introduction to Slow Bee Paralysis virus

Discovered in 1974 in England, Slow Bee Paralysis Virus (SBPV) is characterised by the paralysis of the front two pairs of legs of adult bees *Apis mellifera*, a few days before dying, which occurs approximately 10 days after infection by the virus.

The virus tends to accumulate in the head, jaw and saliva glands as well as the digestive tract and is less prevalent in the hindlegs of the bee. 362 particles of the virus are enough to kill half the infected host. (LD50)

The SBPV genome is approx. 9.5kb long with one ORF containing 2964 amino acids. Two strains labelled 'Rothamsted' and 'Harpenden' and are approx. 83% identical in terms of sequence and 94% homologous in terms of amino acids. Most of the differences are C-U or A-G transitions which are common to RNA viruses.

The SBPV viron is a 30nm icosahedral (20 sided) particle with an RNA genome and 3 major capsid proteins. It is similar to picorna-like mite transferred viruses but is relatively rare. Like Deformed wing virus, V.Destructor virus 1 and sacrood virus, the genome is arranged with the structural proteins being encoded in the N-terminal ORF and other proteins in the C-terminal area.

The virus is associated with, and transmitted to adult bees and pupae by *Varroa destructor* mites. Despite this association, SBPV is rarely detected in bee colonies even if *Varroa* is detected.

SBPV can be asymptomatic in larvae and pupae, but can be detected at this stage. Ultimately SBPV can cause colony collapse disorder and result in total destruction of the hive.

Because SBPV is highly virulent, killing the host bee rapidly before significant replication, it is relatively rare in colonies even those which have a strong *V.destructor* mite presence.

# Specificity

The PCRmax™ qPCR Kit for Slow Bee Paralysis virus (SBPV) genomes is designed for the in vitro quantification of SBPV genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the SBPV genome.

The primers and probe sequences in this kit have 100% homology with a broad range of SBPV sequences based on a comprehensive bioinformatics analysis.

The kit has been developed to detect all subtypes of Slow Bee Paralysis Virus (SBPV) and will detect both the Rothamsted and Harpenden strains.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to [CPtechsupport@coleparmer.com](mailto:CPtechsupport@coleparmer.com) and our bioinformatics team will answer your question.

# Kit contents

- **SBPV specific primer/probe mix (150 reactions BROWN)**  
FAM labelled
- **SBPV positive control template (for Standard curve RED)**
- **Internal extraction control primer/probe mix (150 reactions BROWN)**  
VIC labelled as standard
- **Internal extraction control RNA (150 reactions BLUE)**
- **Endogenous control primer/probe mix (150 reactions BROWN)**  
FAM labelled
- **SBPV/Internal extraction control/endogenous control RT primer mix (150 reactions GREEN)**  
Required for two step protocol only
- **RNase/DNase free water (WHITE)**  
for resuspension of primer/probe mixes
- **Template preparation buffer (YELLOW)**  
for resuspension of internal extraction control template, positive control template and standard curve preparation

## Reagents and equipment to be supplied by the user

### Real-time PCR Instrument

#### RNA extraction kit

This kit is designed to work well with all processes that yield high quality RNA with minimal PCR inhibitors.

#### Lyophilised OneStep 2X RT-qPCR Master Mix

This kit is designed to be compatible with all commercially available OneStep master mix that run with standard cycling conditions.

#### Pipettors and Tips

#### Vortex and centrifuge

#### Thin walled 1.5 ml PCR reaction tubes

## Kit storage and stability

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

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

**PCRmax does not recommend using the kit after the expiry date stated on the pack.**

## Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNase/DNase free water.

## Dynamic range of test

Under optimal PCR conditions PCRmax SBPV detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

## Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period PCRmax detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applied Biosystems Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

## Trademarks

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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. BI, ABI PRISM®, GeneAmp® and MicroAmp® are registered trademarks of the Applied Biosystems (Applied Biosystems Corporation). BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc., The purchase of the PCRmax reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-La Roche Inc.

# Principles of the test

## Real-time PCR

A SBPV specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the SBPV cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled 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

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template.

This can be used to generate a standard curve of SBPV copy number / Cq value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. 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 target SBPV gene 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.

### **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.

A separate RT primer mix and a qPCR primer/probe mix are supplied with this kit to detect the exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the SBPV target cDNA 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.

### **Endogenous control**

To confirm extraction of a valid biological template, a primer and probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel and it is NOT therefore possible to perform a multiplex with the SBPV primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

## Resuspension protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be 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 and probe mix is in the base of the tube and is not spilt 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, vortex each tube thoroughly.

Component - resuspend in water	Volume
<b>Pre-PCR pack</b>	
SBPV primer/probe mix (BROWN)	165 µl
Internal extraction control primer/probe mix (BROWN)	165 µl
<b>SBPV RT primer mix (GREEN)</b>	165 µl
Endogenous control primer/probe mix (BROWN)	165 µl

- 3. 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
<b>Pre-PCR heat-sealed foil</b>	
Internal extraction control RNA (BLUE)	600 µl
<b>Post-PCR heat-sealed foil</b>	
<b>SBPV Positive Control Template (RED) *</b>	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 4µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer per sample.**
- 2. Complete RNA extraction according to the manufacturers protocols.**



# OneStep RT-qPCR detection protocol

A OneStep approach combining the reverse transcription and amplification in a single closed tube is the preferred method.

## For optimum performance and sensitivity.

All pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the One Step 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
Lyophilised OneStep 2X RT-qPCR Master Mix	10 µl
SBPV primer/probe mix (BROWN)	1 µl
Internal extraction control primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	3 µl
<b>Final Volume</b>	<b>15 µl</b>

- 2. For each RNA sample prepare an endogenous control reaction according to the table below (optional):**

This control reaction will provide crucial information regarding the quality of the biological sample.

Component	Volume
Lyophilised OneStep 2X RT-qPCR Master Mix	10 µl
Endogenous control primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
<b>Final Volume</b>	<b>15 µl</b>

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

- 4. Pipette 5µl of RNA template into each well, according to your experimental plate set up.**

For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

5. If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:

Component	Volume
Lyophilised OneStep 2X RT-qPCR Master Mix	10 µl
SBPV primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
<b>Final Volume</b>	<b>15 µl</b>

6. Preparation of standard curve dilution series.

- 1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
- 2) Pipette 10µl of Positive Control Template (RED) into tube 2
- 3) Vortex thoroughly
- 4) Change pipette tip and pipette 10 µl from tube 2 into tube 3
- 5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	$2 \times 10^5$ per µl
Tube 2	$2 \times 10^4$ per µl
Tube 3	$2 \times 10^3$ per µl
Tube 4	$2 \times 10^2$ per µl
Tube 5	20 per µl
Tube 6	2 per µl

7. Pipette 5µl of standard template into each well for the standard curve according to your plate set-up

The final volume in each well is 20µl.

## OneStep RT-qPCR amplification protocol

Amplification conditions using Lyophilised OneStep 2X RT-qPCR Master Mix.

	Step	Time	Temp
	Reverse Transcription	10 min	55 °C
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	<b>DATA COLLECTION *</b>	60 s	60 °C

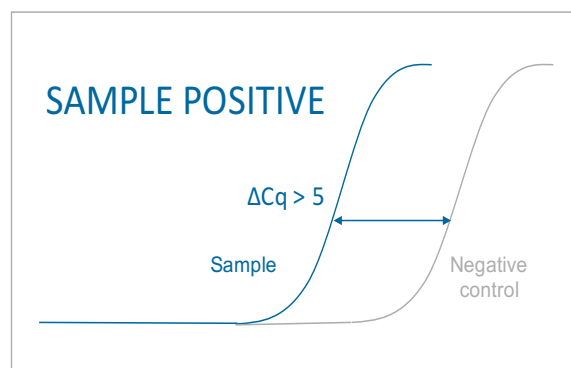
\* Fluorogenic data should be collected during this step through the FAM and VIC channels

# Interpretation of Results

Target (FAM)	Internal control (VIC)	Positive control	Negative control	Interpretation
$\leq 30$	+ / -	+	-	<b>POSITIVE QUANTITATIVE RESULT</b> calculate copy number
$> 30$	+	+	-	<b>POSITIVE QUANTITATIVE RESULT</b> calculate copy number
$> 30$	-	+	-	<b>POSITIVE QUALITATIVE RESULT</b> do not report copy number as this may be due to poor sample extraction
-	+	+	-	<b>NEGATIVE RESULT</b>
+ / -	+ / -	+	$\leq 35$	<b>EXPERIMENT FAILED</b> due to test contamination
+ / -	+ / -	+	$> 35$	*
-	-	+	-	<b>SAMPLE PREPARATION FAILED</b>
+ / -	+ / -	-	+ / -	<b>EXPERIMENT FAILED</b>

Positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

\*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.

**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 RT and PCR reaction and the individual machine settings. Cq values of  $28 \pm 3$  are within the normal range. When amplifying a SBPV 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.

**Endogenous control**

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.