Sleeping disease syndrome - Real Time RNA

BioinGentech Biotechnology

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

OneVetqPCR-realtime™ SDV RNA Real Time PCR Kit is a screening assay for rapid and accurate detection of Sleeping disease syndrome.

Principles of the test:

One Step Bioingentech® qRT-PCR Kits provide components for onestep reverse transcription and quantitative PCR (qRT-PCR) in a convenient format that is compatible with both rapid and standard qPCR cycling conditions. 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 Transcriptase, Recombinant Ribonuclease Inhibitor in an optimized formulation, also include Bioingentech® Taq DNA polymerase and all reagents for an optimized qRTPCR.

The Sleeping disease syndrome specific primer and probe mix are provided in the kit and these can be detected through your real time platform by the 5′ nuclease PCR detection method. During PCR amplification, forward and reverse primers hybridize to the Sleeping disease syndrome target cDNA generated. Fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5-reporter kellú ZZ™ and 3-quencher kurü Zy™ which can be detected through green channel **(see Table 8 and 9).**
To confirm extraction of a valid biological template an Internal control primer and probe mix is included, consists of a DNA probe labeled with a 5-reporter Chods ZX™ and a 3-quencher kurü Zy™ which hybridize inside 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 yellow channel **(see Table 8 and 9).**
Also our RNA Kits include an exogenous source of RNA template that is spiked into the lysis buffer. A separate qPCR primer/probe mix are supplied with this kit to detect an exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which consists of a DNA probe labeled with a 5-dye Chods ZX™ and a 3-quencher kurü Zy™ that during PCR amplification a fluorescence increase can be detected through yellow channel of real time PCR platforms. Successful co-purification and qPCR for the control RNA indicates that PCR inhibitors are not present at a high concentration **(see Table 8 and 9).**
Our kits also include Positive and Negative Control which are details in FAQ section.

Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect Sleeping disease syndrome in real time PCR. This is a possibility relative/absolute quantification or qualitative assay.
Real time PCR is based on fluorogenic dyes. Ct value between 12 – 36 should be taken positive. Value between 36-40 Ct should be taken as marginal positive. Ct 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, cerebrospinal fluid, digestive system, tissue, Heptopancreas, Gills, Pleopods, Cloacal, Egg Yolk, Milk, swabs, Lee, bacterial cultures, cell lines and others. We have available detail procedures for RNA extraction from these samples. All our kits contain all reagents for a really good quality RNA extraction through Trizol method.
We discarded use of affinity columns because a lot report that indicate purification problems due to the lipids present in the biological samples quickly clog the column decreasing its performance.

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)
SDV Positive Control	(1 unit)	(1 unit)	(1 unit)
SDV 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)

* Remember that all our OneVetqPCR-realtime™ SDV RNA Real Time PCR Kits include reagents and procedures for RNA extraction. Also we always can offer you a complete technical support for your different sample type.

Table 2. Instrument Compatibility:

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

For more details you can download a complete compatibility panel from our web site: http://www.bioingentech.com/pdf/Instruments%20Real%20Time.pdf

Procedure:

Please read through the entire procedure before starting.

Before Starting

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

Step 1

Prepare a Master mix according to the reaction 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	10 µL	10 µL	10 µL	10 µL
Primer, Probes and Internal Control Mix	2 µL	2 µL	2 µL	
One qRTPCR Enzyme Mix	2 µL		2 µL	2 µL
Exogenous RNA control Primer and Probes Mix				2 µL
PCR grade Water	4 µL	6 µL	4 µL	4 µL
RNA Sample	2 µL			2 µL
SDV Positive Control		2 µL		
SDV Negative Control			2 µL	
Total Volume	20 µL	20 µL	20 µL	20 µL

Step 2

Place the tubes in a thermal cycler and perform One Step qPCR according to the program outlined in Table 2.

Table 4. Six Step PCR Cycling table

Cycles	Steps	Time	Temp. (°C)
1 Cycle	Reverse Transcription	30 min	50 °C
1 Cycle	Denaturation Step	30 seg	95 °C
40 Cycles	Denaturation	20 seg	95 °C
	Annealing	30 seg	60 °C
	Extension	30 seg	72 °C
1 Cycle	Final Extension	5 min	72 °C
	Hold	-	4 °C

*** Note: Hold step could be optional.**

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**

* Is important mentioned that Ct value over 40 is considered Negative result. If Ct value is in a 12 - 36 range, it must be considered as Positive result. This is depending of the sample initial concentration used for each reaction. You should consider that sample real concentration could be modify by the sample purity when this is quantifier

* For more technical information you must request the quality control for each kits. Also you can request more information writing to our email info@bioingentech.com

2) Quantitative analysis:

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

Average Positive Control Concentration	
SDV	See Quality Control

Standar curve	Preparation series a fresh dilution	Concentration	Copy Number
Tube N°1:	2uL SDV 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

*** Important Note: Don't forget 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 you must include a new dilution tube (Tube N° 8). Note: Final DNA copy number will depend of the DNA concentration (you can see it in 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	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL
Primer, Probes and Internal Control Mix	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL
PCR grade Water	6 µL	6 µL	6 µL	6 µL	6 µL	6 µL	6 µL
Tube N° 1 (Positive Control)	2 µL						
Tube N° 2		2 µL					
Tube N° 3			2 µL				
Tube N° 4				2 µL			
Tube N° 5					2 µL		
Tube N° 6						2 µL	
Tube N° 7							2 µL
Total Volume	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL

2.1.-Assess the Ct value when amplification curve of Standard tube 1, 2, 3, 4, 5, 6 passes the threshold line. However, four tubes are sufficient for standard curve. (tube1-tube4).
2.2.- Calculate quantitative value to compare with Ct value of unknown samples and curve of Standard tube 1, 2, 3, 4, 5, 6.
2.3.- When you visualized result in the Real Time PCR platform you must see just one amplification curve for Positive Control. You must not see an Internal Control amplification curve.

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 fit the regression line. A significant difference in observed Ct values between replicates will lower the R-value.
3.3.- The standard curve slope result should be all 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 kellú ZZ™ dye and should be detected through the Green channel of your real time PCR instrument.
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 Sleeping disease syndrome copy number / Ct value.
Alternatively, the positive control can be used at a single dilution Sleeping disease syndrome on where 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. **Particularly, due to amount of this reagent, you should run a positive control for each 12 samples.** A positive result indicates that the primer and probes for detecting the target Sleeping disease syndrome 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 11)**. Sealing all other samples and negative controls before pipetting the positive control into the positive control well tube.

2.-Internal Control:

The internal control is included in Primer, Probes and Internal Control Mix along to the target pathogen detection. In order to interpreted results, read the yellow channel. The internal control assay uses a Chods ZX™ dye and should be detected through the Yellow channel of your real time PCR instrument and gives a Ct value of 28 (+/-5) depending on the level of sample dilution and concentration. A positive result through the Yellow channel therefore 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 11** data.

3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. **Particularly, due to amount of this reagent, you should run a negative control for each 12 samples.** In this instance the Sleeping disease syndrome Negative Control should be used in place of template. A negative result indicates that the reagents have not become contaminated. If a positive result and Ct value less than 36 is obtained, the results should be analyzed and check if a correct amplification curve was obtained. When you obtain a clear amplification curve you should consider repeat your assay due to probably the sample was contaminated **(see Table 11)**.

* 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:

An exogenous source of RNA template 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.

Amplification of this control cDNA does not interfere with detection of the target cDNA even when present at low copy number. Previous of PCR setup you must add 2 µL (20.000 copies) of undiluted Exogenous RNA Control to the lysis/binding solution concentrate that is used for the RNA isolation.

The Internal control is detected through the Yellow 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, you should include and run one Internal RNA control for each 12 samples. Is not necessary run this control for each sample.

* Exogenous RNA control is just referential and its negative result should not interpreted as fail assay when the sample and internal control gave a positive result (see Table 11).
* You must use quencher and reporter dye to setup your software (see table 8 and 9) and run the corresponding channel.

Table 8. Fluorogenic probes, Channels and Dyes

Channel	Source	Detector	Dyes
Green	470 (Nm)	520 (Nm)	**FAM**, Sybr green1, Fluorescein, Eva green, Alerxa flour 488, **kellú ZZ™**
Yellow	530 (Nm)	550 (Nm)	Joe, Vic, **Hex**, Tet, Cal Fluorgold 540, YaKima Yellow, **Chods ZX™**
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, Aeon Zw™.
Crimson	680 (Nm)	710 (Nm)	Quasar 705, Lightcycler Red 705, Alexa Fluor 680

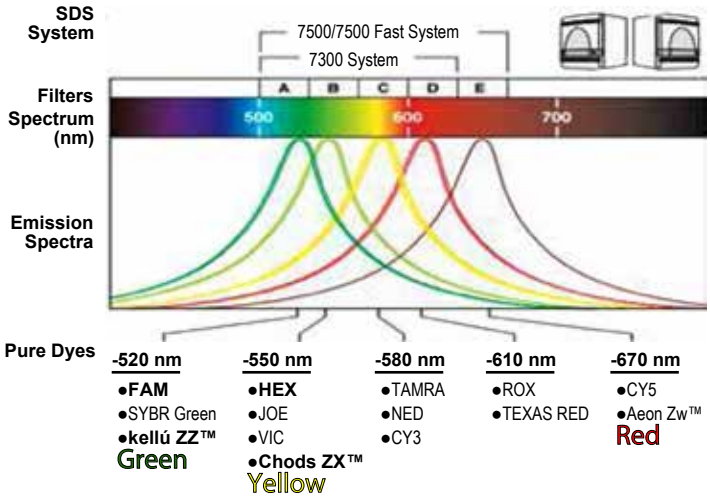

Table 9. Similarity of our fluorophores with HEX and FAM dyes.

	Channel	Dyes	
1.- Positive Control	Green	kellú ZZ™	FAM
2.- Internal Control	Yellow	Chods ZX™	HEX
3.- Sample Target	Green	kellú ZZ™	FAM
4.- Exogenous RNA control	Yellow	Chods ZX™	HEX

Important Note:

Probes for sample and controls mentioned in manuals are just a reference and it not imply that these probes will be the final fluorophores for a purchased kit. The probes combination will be depending of several factor and this information will be available in the Certificate of Analysis when you purchase one of our PCR Kits. kellú ZZ™ and Chods ZX™ fluorophores are just referential.

Is important mentioned that we can develop special request for other pathogens or multiplex detection according client's specifications.

We strongly recommend don't use or combine our products with reagents from another kits or unknown provenance. We can't assure good result 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 10. Store Temperature Kits

	Store temperature	The label temperature
	Shipping temperature	At room temperature

Table 11. 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*
+	+	-	-	+	Exp. Fail
+	+	+	-	+	Exp. Fail
+	-	-	-	-	Exp. Fail
+	-	-	-	+	Exp. Fail
+	-	+	-	-	Exp. Fail
-	+/-	+/-	+/-	+/-	NEGATIVE

* Sometimes amplification curves for Negative or Internal control with Ct < 30 is generate, but it's not necessary a Positive result. You should see and determinate if is a sigmoid curve. If the amplification curve isn't sigmoid you should consider as negative result.

Table 12. Other Products

Products		Code
Bioingentech - Genomic RNA Purification Kit	50 test	PU-A004
Bioingentech - Genomic RNA Purification Kit	100 test	PU-A005
Bioingentech - Genomic RNA Purification Kit	150 test	PU-A006
OneVetqPCR-realtime™ SDV Sleeping disease syndrome Real Time 50 tests (Ready to use kit) / Cat. No: Oneq-V274-50R		
OneVetqPCR-realtime™ SDV Sleeping disease syndrome Real Time 100 tests (Ready to use kit) / Cat. No: Oneq-V274-100R		
OneVetqPCR-realtime™ SDV Sleeping disease syndrome Real Time 150 tests (Ready to use kit) / Cat. No: Oneq-V274-150R		

Table 13. 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 value between 12 – 36 should be taken positive. Value between 36-40 Ct should be taken as marginal positive. Ct above 40 must be considered as negative.
Controls included	Internal control, Internal RNA extraction control, Positive control and Negative control
Channels	Kellú / FAM Green channel detect pathogen amplicons Chods / HEX Yellow channel detect internal control amplicons. Kellú / FAM Green channel detect Positive Control. Chods / HEX Yellow channel detect Exogenous RNA control.