

## Streptococcus phocae - Real time

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

VetqPCR-realtime™ S. phocae Real Time PCR Kit is a screening assay for the detection of Streptococcus phocae.

### Principles of the test

The Streptococcus phocae specific primer and probe mix are provided and this can be detected through the yellow channel.

During PCR amplification, forward and reverse primers hybridize to the Streptococcus phocae. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5-dye Chods ZX™ and 3-quencher kurü Zy™. Internal control consists of a DNA probe labeled with a 5-dye kellü ZZ™ and a 3-quencher kurü Zy™.

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 realtime PCR platforms.

### Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect Streptococcus phocae in real time PCR. This is a possibility absolute quantification or qualitative assay.

Real time PCR is based on fluorogenic dyes. Up to 36 Ct should be taken positive. Value between 36-40 Ct should be taken as marginal positive.

This kit needs DNA which can be isolated from blood, serum, faeces, respiratory fluid, digestive system, tissue and others.

See (PU-A001, PU-A002 & PU-A003) included in advanced formats.

### Kit Components

Pulse-spin each tube in a centrifuge before opening.  
Completely thaw the components of the kit prior to use.  
Homogenize the solutions for 5 seconds prior to pipetting.

| Reactions                          | 50 test  | 100 test | 150 test |
|------------------------------------|----------|----------|----------|
| VetqPCR-realtime™ S.phocae         | (1 vial) | (1 vial) | (1 vial) |
| VetqPCR-realtime™ Internal Control | (1 vial) | (1 vial) | (1 vial) |
| PCR Grade water                    | (1 vial) | (1 vial) | (1 vial) |
| S.phocae Positive control          | (1 vial) | (1 vial) | (1 vial) |
| S.phocae Negative Control          | (1 vial) | (1 vial) | (1 vial) |
| Mineral Oil                        | (1 vial) | (1 vial) | (1 vial) |

### Instrument Compatibility in:

**\* Ask us for information.**

|                     |                             |
|---------------------|-----------------------------|
| * 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            |
| * ExiCycler™ 96     | * RotorGeneQ                |
| * iQ5 & MyiQ Cycler | * SLAN® Real-Time PCR       |
| * Illumina Eco      | * Smartcycles II            |
| * LightCycler Nano  | * Applied 7300 and 7500     |

### Procedure:

Please read through the entire procedure before starting.

### STEP 1

#### Preparation of S. phocae Mixture

1) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 1. The final reaction volume should be 13.5 µL

### Notes:

- Run a positive control, a negative control, and an internal control for each 12 samples.
- The mineral oil is necessary, only when using a thermalcycler that employs a top heating method.

Table 1. Reaction components for PCR

|                                    | Sample | Positive control | Negative control | Internal control |
|------------------------------------|--------|------------------|------------------|------------------|
| VetqPCR-realtime™ S.phocae         | 5.5 µL | 5.5 µL           | 5.5 µL           |                  |
| VetqPCR-realtime™ Internal Control |        |                  |                  | 5.5 µL           |
| PCR Grade water                    | 6µL    | 6µL              | 6µL              | 6µL              |
| DNA isolated from the sample       | 2µL    |                  |                  | 2µL              |
| S.phocae Positive control          |        | 2µL              |                  |                  |
| S.phocae Negative Control          |        |                  | 2µL              |                  |
| Total Volumen                      | 13.5µL | 13.5µL           | 13.5µL           | 13.5µL           |

## Visual explanation:

### Sample:



2µl DNA isolated from the sample  
6µl PCR Grade water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Positive Control:



2µl S. phocae Positive control  
6µl PCR Grade water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Negative Control:



2µl S. phocae Negative control  
6µl PCR Grade water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Internal Control:



2µl DNA isolated from the sample  
6µl PCR Grade water  
5.5µl VetqPCR-realtime™ Internal Control

**Total: 13,5 µL**

### Observation:

homogenize solution in each tube during 10 seconds.

Place the tubes in a Instrument and perform amplification according to the program outlined in Table 2.

**Table 2. Real Time cycling parameters**

| PCR cycle   |                      | Temp. | Time       |
|-------------|----------------------|-------|------------|
| x 1 cycles  | Initial Denaturation | 94°C  | 2 min.     |
| x 45 cycles | Denaturation         | 95°C  | 15 seconds |
|             | Annealing            | 60°C  | 60 seconds |

## Step 3

Once the program will be finished one can see the graphics. The negative control should run along with the bottom and positive control must give a curve in the software graphics. Use your software to analyse the results.

### Interpretation of the test

#### 1) Qualitative analysis:

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

Ct value result

|               |               |
|---------------|---------------|
| > 40 negative | ≤ 40 positive |
|---------------|---------------|

#### 2) Quantitative analysis:

**Table 3. Preparation of standard curve dilution series. S. phocae positive control:**

|  |                     |
|--|---------------------|
| Average Positive Control Concentration |                     |
| <b>S. phocae</b>                       | See quality control |

| Standar curve | Preparation series a fresh dilution                               | Concentration       | Copy Number         |
|---------------|---|---------------------|---------------------|
| Tube N°1:     | 2uL S. phocae Positive Control (0,3 ng/µl) + 18uL PCR Grade Water | See quality control | See quality control |
| Tube N°2:     | 2uL Tube N°1 + 18uL PCR Grade Water                               | See quality control | See quality control |
| Tube N°3:     | 2uL Tube N°2 + 18uL PCR Grade Water                               | See quality control | See quality control |
| Tube N°4:     | 2uL Tube N°3 + 18uL PCR Grade Water                               | See quality control | See quality control |
| Tube N°5:     | 2uL Tube N°4 + 18uL PCR Grade Water                               | See quality control | See quality control |
| Tube N°6:     | 2uL Tube N°5 + 18uL PCR Grade Water                               | See quality control | See quality control |
| Tube N°7:     | 2uL Tube N°6 + 18uL PCR Grade Water                               | See quality control | See quality control |

Homogenize tube.

\* We will send a Quality Control report for each purchase  
\*\* If you want to obtain 13 DNA copies you must include a new dilution tube (Tube N° 8)

Table 4. Reaction components for Real Time PCR standar curve.

|                   | Tube A | Tube B | Tube C | Tube D | Tube E | Tube F | Tube G |
|-------------------|--------|--------|--------|--------|--------|--------|--------|
| VetqPCR S. phocae | 5.5 µl | 5.5 µl | 5.5 µl | 5.5 µl | 5.5 µl | 5.5 µl | 5.5 µl |
| PCR Grade Water   | 6.0 µl | 6.0 µl | 6.0 µl | 6.0 µl | 6.0 µl | 6.0 µl | 6.0 µl |
| Tube N°1          | 2.0 µl |        |        |        |        |        |        |
| Tube N°2          |        | 2.0 µl |        |        |        |        |        |
| Tube N°3          |        |        | 2.0 µl |        |        |        |        |
| Tube N°4          |        |        |        | 2.0 µl |        |        |        |
| Tube N°5          |        |        |        |        | 2.0 µl |        |        |
| Tube N°6          |        |        |        |        |        | 2.0 µl |        |
| Tube N°7          |        |        |        |        |        |        | 2.0 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 is 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.

### 3) Test validation:

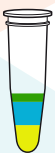
3.1.- Each Ct value standard should be as follows.  
Standard 1 > Standard 2 > Standard 3 > Standard 4 > Standard 5 > Standard 6.

3.2.- R-value of standard curve should be 0.900~0.999.

3.3.- The standard result should be all negative.

## Visual explanation:

### Tube A



2.0µl Tube N°1 (Positive Control)  
6.0µl PCR Grade Water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Tube B



2.0µl Tube N°2  
6.0µl PCR Grade Water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Tube C



2.0µl Tube N°3  
6.0µl PCR Grade Water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Tube D



2.0µl Tube N°4  
6.0µl PCR Grade Water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Tube E



2.0µl Tube N°5  
6.0µl PCR Grade Water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Tube F



2.0µl Tube N°6  
6.0µl PCR Grade Water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

### Tube G



2.0µl Tube N°7  
6.0µl PCR Grade Water  
5.5µl VetqPCR-realtime™ S. phocae

**Total: 13,5 µL**

## FAQ:

### 1.-Positive control:

The positive control assay uses a Chods ZX™ dye and should be detected through the Yellow 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 Streptococcus phocae copy number / CT value. Alternatively the positive control can be used at a single dilution Streptococcus phocae 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. A positive result indicates that the primer and probes for detecting the target Streptococcus phocae gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. sealing all other samples and negative controls before pipetting the positive control into the positive control well tube.

### 2.-Internal Control:

The internal control assay uses a **kellú ZZ™** dye and should be detected through the **Green** channel of your real time PCR instrument. A positive result through the **Green** channel therefore indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the **Green** channel the results should be ignored and the test repeated.

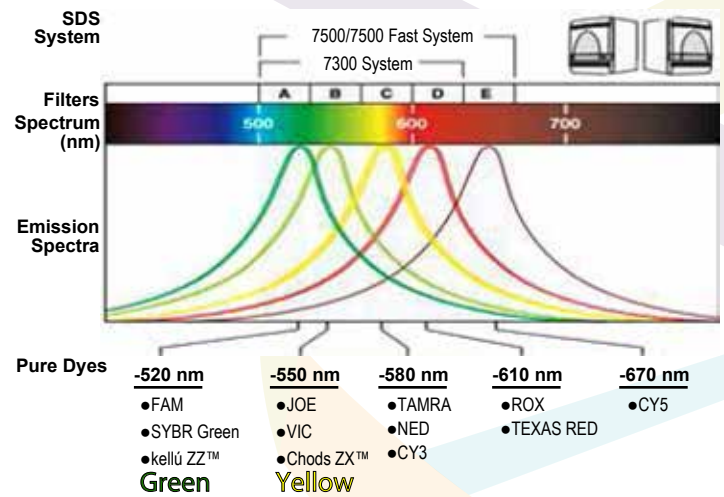
### 3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. In this instance the PCR grade water should be used in place of template. A negative result indicates that the reagents have not become contaminated. If a positive result (CT value less than 37) is obtained the results should be ignored and the test repeated.

You must use quencher and reporter dye to setup your software (see table 2) and run the following channel:

Table 5. Report Dye channel selection chart

| Channel | Source   | Detector | Dyes   |
|---------|----------|----------|--|
| Green   | 470 (Nm) | 520 (Nm) | FAM, Sybr green1, Fluorescein, Eva green, Alerxa flour 488, <b>kellú ZZ™</b> |
| Yellow  | 530 (Nm) | 550 (Nm) | Joe, Vic, Hex, Tet, Cal Fluorgold 540, YaKima Yellow, <b>Chods ZX™</b>       |
| 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                             |



### Similarity

Similarity of our fluorophores with HEX and FAM.

|            |     |
|------------|-----|
| •kellú ZZ™ | FAM |
| •Chods ZX™ | HEX |

### 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.

|  |                      |                       |
|--|----------------------|-----------------------|
|  | Store temperature    | The label temperature |
|  | Shipping temperature | At room temperature   |

Table 6. Interpretation of Results

| Target | Internal Control | Negative Control | Positive Control | Interpretation  |
|--------|------------------|------------------|------------------|-----------------|
| +ive   | +ive             | -ive             | +ive             | +ive            |
| +ive   | -ive             | -ive             | +ive             | +ive            |
| +ive   | +ive             | +ive             | +ive             | *               |
| +ive   | -ive             | +ive             | +ive             | *               |
| -ive   | +ive             | -ive or +ive     | +ive             | -ive            |
| -ive   | -ive             | -ive or +ive     | -ive             | Experiment fail |
| -ive   | +ive             | -ive or +ive     | -ive             | Experiment fail |

Table 7. Products

| Products  | Code     |         |
|---|----------|---------|
| Bioingentech - Genomic DNA Purification Kit   | 50 test  | PU-A001 |
| Bioingentech - Genomic DNA Purification Kit   | 100 test | PU-A002 |
| Bioingentech - Genomic DNA Purification Kit   | 150 test | PU-A003 |
| VetqPCR-realttime™ S. phocae Streptococcus phocae Real Time 50 tests (Ready to use kit) / Cat. No: RTq-V247-50D   |          |         |
| VetqPCR-realttime™ S. phocae Streptococcus phocae Real Time 100 tests (Ready to use kit) / Cat. No: RTq-V247-100D |          |         |
| VetqPCR-realttime™ S. phocae Streptococcus phocae Real Time 150 tests (Ready to use kit) / Cat. No: RTq-V247-150D |          |         |