

Taura Syndrome Virus (TSV) Real Time RT-PCR Kit

Cat. No.: AR-0200-01

For Use with LightCycler1.0/LightCycler2.0 Real Time PCR Systems

1. Intended Use

TSV real time RT-PCR kit is used for the detection of Taura syndrome virus (TSV) in gill or muscle samples of Shrimp by real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

The single-stranded genomic RNA of Taura syndrome virus (TSV) is 10205 nucleotides in length, excluding the 3' poly(A) tail, and contains two large open reading frames (ORFs) that are separated by an intergenic region of 207 nucleotides. The ORFs are flanked by a 377 nucleotide 5' untranslated region (UTR) and a 226 nucleotide 3' UTR followed by a poly(A) tail. While the severity of this bout of Taura Syndrome is somewhat atypical, the shrimp farmer's experiences in coping with the disease underscore the devastating impact that illness can have on the larval and growout production of marine shrimp, and the need for practical tools for disease management. TSV real time RT-PCR kit contains a specific ready-to-use system for the detection of TSV by Reverse Transcription Polymerase Chain Reaction (RT-PCR) in the real-time PCR system. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the TSV RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of polymerase chain reaction (PCR). Fluorescence is emitted and measured by the real time systems' optical unit during PCR. The detection of amplified TSV cDNA fragment is performed in fluorimeter channel FAM with the fluorescent quencher BHQ1. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). An external positive control (1x10⁶copies/ml) contained, allows the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

4. Kit Contents

Ref.	Type of reagent	Presentation	25rxns
1	TSV Super Mix	1 vial, 350µl	
2	RT-PCR Enzyme Mix	1 vial, 28µl	
3	Molecular Grade Water	1 vial, 400µl	
4	Internal Control	1 vial, 30µl	
5	TSV Positive Control(1x10 ⁶ copies/ml)	1 vial, 30µl	

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "ependorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- RNA extraction kit
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

7. Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
 - This assay needs to be carried out by skilled personnel.
 - Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
 - This assay needs to be run according to Good Laboratory Practice.
 - Do not use the kit after its expiration date.
 - Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
 - Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
 - Prepare quickly the Reaction mix on ice or in the cooling block.
 - Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
 - Pipets, vials and other working materials should not circulate among working units.
 - Use always sterile pipette tips with filters.
 - Wear separate coats and gloves in each area.
 - Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
 - Avoid aerosols

Sample Collection, Storage and transport

- Collected samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 RNA-Extraction

RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction, please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
RNA Isolation Kit	ME-0010/ME-0012	ZJ Biotech
QIAamp Viral RNA Mini extraction Kit (50)	52904	QIAGEN

9.2 Internal Control and Positive Control

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.

Add the internal control (IC) 1µl/rxn and the result will be shown in the HEX/VIC/JOE.

Attention:

It is necessary to dilute the positive control supplied in the kit to 10⁷ copies/ml by 10 times with molecular grade water before detection, and close the tube immediately then vortex for 10 seconds.

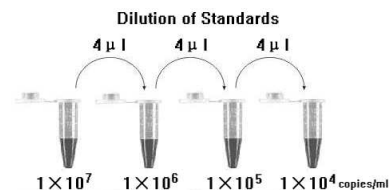
9.3 Quantitation

The kit can be used for **quantitative** or **qualitative** real-time RT-PCR.

For performance of quantitative real-time PCR, standard dilution must be prepared first as follows. Molecular Grade Water is used for dilution.

Dilution is not needed for performance of qualitative real-time PCR.

Take positive control (1x10 copies/ml) as the starting high standard in the first tube. Respectively pipette 36ul of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



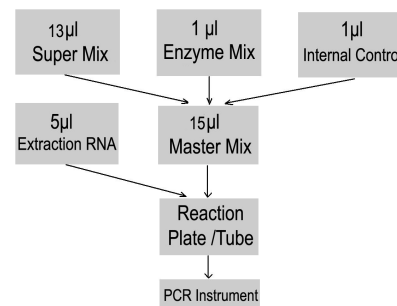
To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standards with specification of the corresponding concentrations.

Attention:

- Mix thoroughly before next transfer.
- The positive control contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

9.4 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



✘ PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

- The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
- Pipet 15µl Master Mix with micropipets of sterile filter tips to each of the Real time PCR reaction plate/tubes. Separately add 5µl RNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
- Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- Perform the following protocol in the instrument:

45°C for 10 min, 1 cycle; 95°C for 15 min, 1 cycle; 95°C for 5 sec, 60°C for 30sec, 40 cycles.

Fluorescence is measured at 60°C, and FAM and HEX/VIC/JOE channels should be chosen.

10. Baseline setting: just above the maximum level of molecular grade water.

11. Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12. Quality control: The crossing point value of molecular grade water and positive control in FAM channel shows blank and ≤35 respectively; The crossing point value of internal control in HEX/VIC/JOE channel shows 25-33; Correlation coefficient of standard curve should be ≤ -0.98, otherwise the result is invalid.

13. Data Analysis and Interpretation

The following results are possible:

- The crossing point value in channel FAM shows ≤38. **The result is positive: The sample contains Taura syndrome virus RNA.**
- The crossing point value in channel FAM shows 38-40, please repeat again. **If the result still shows 38-40, it can be considered negative.**
- In channel FAM no signal is detected, at the same time, a HEX/VIC/JOE signal from the Internal Control appears. **The sample does not contain any Taura syndrome virus RNA. It can be considered negative.**
- Neither in channel FAM nor in channel HEX/VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the RT-PCR reaction.