DATA SHEET

White Spot Syndrome Virus (WSSV) Real Time PCR Kit

1. Intended Use

White Spot Syndrome Virus (WSSV) real time PCR kit is used for the detection of White Spot Syndrome Virus in gill or muscle samples of Shrimp by using 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

White spot virus (WSV) or white spot syndrome virus (WSSV) is a presently unclassified rod-shaped to obovate, enveloped double-stranded DNA virus with a single filamentous appendage. The cellular location is nuclear and the genome is large at approximately 290 kbp. White spot disease (WSD) has been recorded from most Asian countries were penaeid shrimp are pond reared. Original outbreaks were reported from the People's Republic of China in 1993 and they spread rapidly thereafter to Japan, Taipei China and the rest of Asia, but not Australia. Since early 1999, it has been widely reported from shrimp farms in the southern United States of America, Central America and northern South America. Disease outbreaks may occur at all seasons and at all phases of pond rearing, but they seem to be favored by widely fluctuating environmental conditions.

White Spot Syndrome Virus real time PCR kit contains a specific ready-to-use system for the detection of the White Spot Syndrome Virus by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the White Spot Syndrome Virus DNA. Fluorescence is emitted and measured by the real time systems´ optical unit. The detection of amplified White Spot Syndrome Virus DNA fragment is performed in fluorimeter **channel 530nm** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and gill or muscle samples are used for the extraction of the DNA. In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560nm fluorescence of the internal control (IC). An external positive control (1×10^7copies/ml) contained, allows the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

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4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	1 vial, 1.8ml
2	WSSV Reaction Mix	1 vial, 450µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control (IC)	1 vial, 30µl
6	WSSV Positive Control (1×10^7copies/ml)	1 vial, 30µl

Analysis sensitivity: 1×10^3copies/ml; LOQ: 2×10^3～1×10^8copies/ml

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 "eppendorf" 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, and smoke in laboratory.
- Avoid aerosols

8. 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 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

1) Take 50mg sample to a tube, add 50µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

2) Incubate the tube for 10 minutes at 100°C.

3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

Attention:

A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination in case the sample is positive.

B. The extraction sample should be used in 3 hours or stored at -20°C for one month.

C. DNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For DNA extraction, please comply with the manufacturer's instructions.

9.2 Internal 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 560nm.

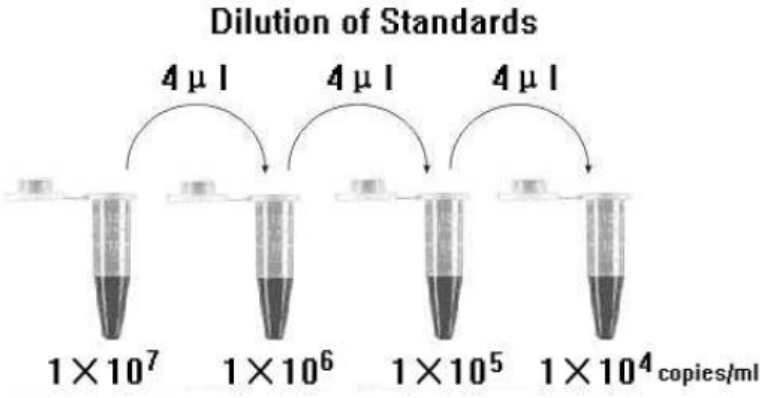
9.3 Quantitation

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

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

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

Take positive control (1×10^7copies/ml) as the starting high standard in the first tube. Respectively pipette **36ul** 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 standard with specification of the corresponding concentrations.

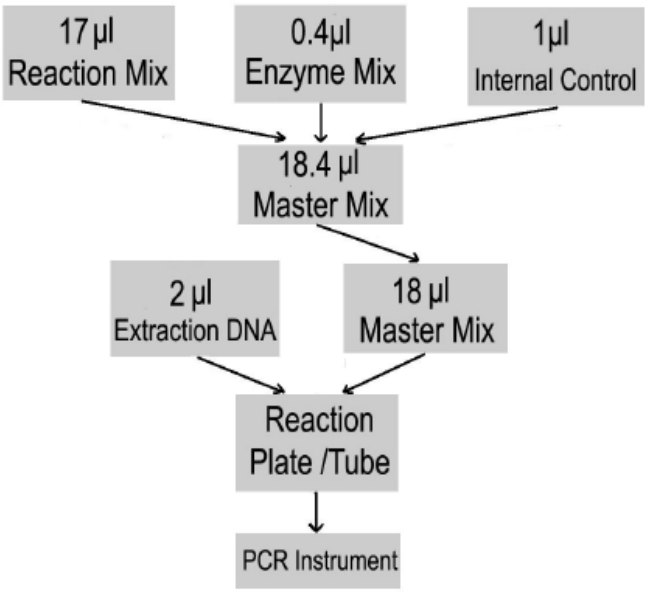
Attention:

A. Mix thoroughly before next transfer.

B. The positive control contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

9.4 PCR Protocol

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



※PCR system without 560nm channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Reaction 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.
2) Pipet **18µl Master** Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tubes. Separately add **2µl** DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

37°C for 2min	1cycle
94°C for 2min	1cycle
93°C for 5sec, 60°C for 30sec (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
530nm	Target Nucleic Acid
560nm	IC

10.Threshold setting: Choose **Arithmetic** as back ground and **none** as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

11.Calabration 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: Negative control, positive control, internal control and QS curve must be performed correctly, otherwise the sample results is invalid.

Channel / Control	Crossing point value	
	530nm	560nm
Molecular Grade Water	Blank	25~33
Positive Control(qualitative assay)	≤35	——
QS（quantitative detection）	Correlation coefficient of QS curve≤－0.98	

13. Data Analysis and Interpretation

The following results are possible:

	Crossing point value		Result Analysis
	530nm	560nm	
1#	Blank	25~33	Below the detection limit or negative
2#	≤35	——	Positive; and the software displays the quantitative value
3#	35～40	25~33	Re-test; If it is still 35~40, report as 1#
4#	Blank	Blank	PCR Inhibition; No diagnosis can be concluded.