

# REAL-TIME PCR KIT FOR WHITE SPOT SYNDROME VIRUS (WSSV) DETECTION

*-Intended for molecular detection of WSSV in shrimp*

**CATALOG NUMBER: DIG-WSSVR-25**

For Research Use Only

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## Kit Contents

Components	Volume
Master Mix with ROX (2X)	300 µL×1 vial
WSSV Primer & Probe Mix [ready to use]	50 µL × 1 vial
Internal control Primer probe mix [ready to use]	50 µL × 1 vial
WSSV Positive control	60 µL × 1 vial
Nuclease free water	200 µL × 1 vial

## Storage:

The contents of the kit should be stored at –20 °C and are stable until the expiration date. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. The master mix may be frozen at -20 °C for long term storage. Once thawed, it should be stored at 4 °C and used within 6 months.

## Precaution before use

1. Before use, make sure the master mix is evenly mixed by gently inverting the tube several times without creating bubbles. Uneven reagent mixing will result in inadequate reactivity. Do not mix by vortexing.
2. When stored frozen at -20 °C, master mix may precipitate. To dissolve the precipitate completely, warm by hand or let stand at room temperature briefly, then invert the tube several times. Make sure the reagents are evenly mixed before use.
3. Place reagents on ice immediately after it has thawed.
4. Use fresh disposable tips to minimize potential cross-contamination between samples when preparing reaction mixtures or dispensing aliquots.

## Equipment and materials to be supplied by User

- PCR tubes or plates designed specifically for the qPCR instrument
- Micropipette and tips (sterile, with filter)
- Real-time PCR thermal cycler
- Ice bucket

## Introduction:

The aquaculture industry is one of the fastest-growing food production sectors and constitutes an important economic activity in developing countries. However, climate change and the emergence of pathogens have negatively impacted the aquaculture industry over the past 20 years. Infectious diseases caused by viruses, bacteria, fungi, and microsporidia pose a serious threat to shrimp aquaculture. Among them, viral diseases are by far the most difficult to manage.

## Product Description:

The Real-time PCR kit for White Spot Syndrome Virus (WSSV) is intended for the molecular detection of WSSV in shrimp. WSSV is rapidly replicating and extremely virulent shrimp pathogen, has emerged globally as one of the most prevalent and widespread belonging to genus *whispovirus* (White Spot) which is the only genus in the family *Nimaviridae*. It is responsible for causing white spot syndrome in a wide range of crustacean host. WSSV-infected shrimp may rapidly develop white spots (ranging from 0.5–3.0 mm in diameter) on the exoskeleton, appendages and inside the epidermis. Other signs of WSSV include lethargy, sudden reduction in food consumption, red discoloration of body and appendages and a loose cuticle. This assay allows rapid detection of WSSV which involves the use of target and internal control sets of primers in a single tube by using real-time polymerase chain reaction (PCR).

## Protocol:

- For all reagents preparation, wear appropriate gloves.
- A Master Mix is prepared in a tube by combining a 2X Master Mix Reagent and Primer & Probe Mix with water (protect from light).

Reagents	Vol. for 1 rxn (µL)
Water	4.8
WSSV Master Mix with ROX (2X)	10
WSSV Primer & Probe Mix [ready to use]	1.6
Internal control Primer probe mix [ready to use]	1.6
Template DNA/Positive control	2.0
Total	20

TaqMan® probe reporter dyes and quenchers

Detector Name	Reporter	Quencher
WSSV Target	FAM <sup>TM</sup> dye	None
Internal Control	VIC <sup>TM</sup> dye	None

- After mixing thoroughly, 18µl volumes of the Master Mix are quickly dispensed into the plate/Strip
- Add 2µl of DNA test samples and controls.
- Pulse spin tubes at 3000rpm.
- Keep the plate/strips/tubes in thermal cycler

Stages		Cycles	Temperature	Time
Template denaturation	Holding stage	1	95 °C	30 sec
Amplification	Cycling stage	40	95 °C 60 °C	5 sec 31 sec

- After the completion of the reaction, observe the Ct value of the samples and controls.

## Specificity

The primers have been designed for specific and *in vitro* detection of WSSV. This kit has high priming efficiency of  $\geq 95\%$  and can detect less than 10 copies of target template.

## Expected Ct value: Ct < 38 – Positive

- In positive control (PC), only target shows Ct value less than 38 whereas no Ct value is observed in endogenous control gene of PC.
- For samples, both target and endogenous control gene shows Ct value less than 38 which is considered as positive.

## Data interpretation:

	Outcome 1	Outcome 2	Outcome 3	Outcome 4
<b>Test sample</b>	Positive	Negative	Positive	Negative
<b>Positive control</b>	Positive	Positive	Positive	Negative
<b>Negative control</b>	Negative	Negative	Positive	Negative
<b>Internal control</b>	Positive	Positive	Positive	Negative
<b>Interpretation</b>	Infection detected	Infection not detected	PCR Contamination	Experiment failed

### **Troubleshooting guide:**

- Setup errors
  - Mix well all reagents before every use.
  - Make master mixes whenever possible as this reduces pipetting errors.
- Fluorescence in negative control
  - There is a possibility that template DNA might be added in the NTC reaction.
  - Contamination in reagents-Aliquot the reagents before setting up the reaction.
  - Random contamination-
    - Clean the work area with 10% bleach and nuclease-free water
    - Use separate working areas for PCR mix preparation, template addition, and performing the PCR reactions.
- Unexpected data values
  - Samples incorrectly labelled-Rerun samples or plate using extra caution when loading
  - Inhibitors in sample-Run a dilution of samples (inhibitors will be diluted and may result in lower C<sub>q</sub> for diluted material)
- C<sub>q</sub> is much earlier than anticipated- Genomic DNA is contaminated with RNA
  - Test assay performance against carefully quantified controls
- Jagged signal throughout amplification plot- Poor amplification or weak probe signal
  - Ensure that a sufficient amount of probe is used so that background noise isn't amplified
  - Try a fresh batch of probe
  - Mix primer/probe/master solution thoroughly during reaction set up
- Rough amplification curves
  - Improper storage of primers and probes can cause them to degrade and lose specificity, which in turn affects the reaction efficiency.

#### **TECHNICAL ASSISTANCE**

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