Innocreate Bioscience® WSSV QD Kit （192 rxn.）

Cat. No. :P01-01-01-192

The ***Innocreate Bioscience*® WSSV QD Kit** constitutes a ready-to-use dual-fluorescence system for the detection of white spot syndrome virus (WSSV) in shrimp. Using real-time polymerase chain reaction (qPCR), the ***Innocreate Bioscience*® WSSV QD Kit** is designed to detect and measure the WSSV and the shrimp genomic DNA in the same reaction. The ratio of WSSV and the shrimp genomic DNA are used for the diagnosis of WSSV infections.

1. Materials and Equipment

1.1 Kit Contents

The ***Innocreate Bioscience*® WSSV QD Kit** contains reagents for 192 reactions. Components are listed as the table below:

Cap Color	Component Name	Volume	Tube
Blue	QD reagent set (QD)	400 µl	1
Red	2X Reaction Master Mix (RM)	1000 µl	2
Yellow	WSSV QD Standard (STD) (10^7 copies/µl)	100 µl	1
Green	Yeast tRNA	1500 µl	1
Orange	Distilled Water DNase, RNase Free	1400 µl	1

1.2 Storage

The guidelines below for storing ***Innocreate Bioscience*® WSSV QD Kit**:

- Upon receipt, store the QD reagent set at -20℃ and to be protected from light.
- Store the WSSV QD Standard at -20℃
- Store the 2X Reaction Master Mix at -20℃, and protect RM reagent from light. Excessive exposure to light may affect the fluorescent reference dye. When Master Mix thaw out, it can be stored and protected from light at 2~8℃ for up to 2 months.
- Do not freeze-thaw ***Innocreate Bioscience*® WSSV QD Kit** in excess of 5 times.

1.3 Equipment and Materials Not Included

The following tables include equipment and materials for using the ***Innocreate Bioscience***® **WSSV QD Kit**. Unless otherwise noted, many of the items listed are available from major laboratory suppliers (MLS).

Instruments	**Source**
Applied Biosystems 7900HT Fast Real-Time PCR System	Applied Biosystems
Applied Biosystems 7300/7500 Real-Time PCR System	
Bio-Rad CFX Real-Time PCR Detection System	Bio-Rad
Desktop centrifuge with rotor for 2 ml reaction tubes	MLS
Bench top micro centrifuge (for spin down 0.2ml strips)	MLS
Vortex mixer	MLS
Pipettes (adjustable) L1000 / L200 / L20 /L10 /L2 / Multichannel L8-20 (RAININ)	MLS (RAININ LTS systems is recommend)

Attention: A valid calibration of the pure dyes (*Pure Spectra Component File*) and the background (*Background Component File*) is necessary when putting the instruments into operation.

Materials	**Source**
High-Throughput Setup	
MicroAmp® Optical 96-Well Reaction Plate with Barcode (20 plates)	Applied Biosystems (PN 4306737)
MicroAmp® Optical Adhesive Film (100 covers)	Applied Biosystems (PN 4311971)
96-Well Reaction Plate	MLS
Mid-to-Low-Throughput Setup	
MicroAmp® 8-Tube Strip, 0.2 ml (125strips)	Applied Biosystems (N8010580)
MicroAmp® Optical 8-Cap Strip (300 strips)	Applied Biosystems (PN 4323032)
Optical 8-Tube Strip	MLS
Other Materials	
Disposable powder-free gloves	MLS
Pipet tips, aerosol resistant GP-L10F/GP-L250F/GP-L1000F	MLS (RAININ LTS Filter Tips is recommend)
RNase-free, sterile-filtered water	MLS
Sterile microcentrifuge tubes, 1.5ml / 2.0 ml	MLS

2. Definitions of Terms

This protocol assumes these definitions:

- **Amplification** – The process of increasing the amount of a specific DNA sequence.
- **Polymerase Chain Reaction (PCR)** – Technology used to amplify a DNA sequence.
- **QD reagent set (QD)** – QD reagent contains specific primers and probes to detect both WSSV and shrimp DNA.
- **2X Reaction Master Mix (RM)** – A common reagent contains the polymerase enzyme that initiates PCR in the presence of the necessary assay and DNA sample.
- **WSSV QD Standard (STD)** – A modified plasmid contains one copy of WSSV target sequence and one copy of shrimp target sequence.
- **Yeast tRNA** – A reagent for quantitative recovery of small amounts of nucleic acids in dilute solutions, use for dilution WSSV QD Standard.
- **Premix solution** – A solution prepared by operator that contains 2X Reaction Master Mix (RM) and QD reagent set (QD).
- **Unknown sample** – A DNA sample extracted from shrimp to detect WSSV.
- **No-template control (NTC)** – It is highly recommended the at least one no-template control is included in one run to detect contamination during reaction setup.
- **Positive control** – Known shrimp sample contains WSSV. Monitors for the detection of WSSV and shrimp target. Target signal not detected in a positive control well indicates a pipetting error or a problem with amplification. A positive control is optional and not recommended because it may cause cross-contamination.
- **Negative control** – Known shrimp sample does not contain WSSV. Monitors to check the diagnosis of WSSV infections. A negative control is optional and not recommended because it may cause cross-contamination.

3. General Precautions

The user should always pay attention to the following:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in a cooling block.

4. DNA Extraction

4.1 Tissue for DNA Extraction

~20 mg shrimp tissue from four different tissues (either one of the following is fine)

- pleopods of live juvenile to subadult shrimp
- postlarvae 11 upwards [PL11 up] with removed heads
- whole PL10
- 100 µl haemolymph

4.2 DNA Extraction Methods

- TANBEAD Tissue Total DNA Kit (6T2A46) for shrimp tissue DNA extraction.
- DNA extraction methods described in "Manual of Diagnostic Tests for Aquatic Animals - White spot disease" (4.3.1.2.4.1).
- Other commercial DNA extraction kits.

4.3 DNA Quality Check

To evaluate DNA purity, measure absorbance from 260nm to 280nm. Good-quality DNA will have an A260/A280 ratio of 1.6–1.8.

5. Preparation of qPCR Reactions

5.1 Sample DNA Preparation

Dilute the sample DNA to the appropriate concentration (0.1~1 ng/μl) to prevent the PCR inhibition effect. Add at least 2 μl sample DNA to nuclease free water to minimize pipetting errors, then briefly vortex. Use 2 μl diluted sample DNA for each QPCR reaction.

5.2 WSSV QD Standard Preparation

Dilute the WSSV QD Standard (10^7 copies/μl) with Yeast tRNA (provided in the kit). To get 10^6 copies/μl, add 10 μl WSSV QD Standard into 90 μl Yeast tRNA and mix well. Use the same method to get standards for 10^5- 10^2 copies/μl. Use at least 5 standards (10^6-10^2) to generate the standard curve. Use 2 μl standard for each standard reaction.

5.3 Premix Preparation

Determine the total number of reactions needed for the respective number of replicates for each of the following reactions. Two or three replicates are recommended:

- WSSV QD Standards (at least 5 standards)
- Each dilution of every sample DNA
- Negative control (NTC)

Create the Premix Solution and mix thoroughly according to the following table:

Component	Volume for 1 reaction
QD reagent set (QD)	2 μl
2X Reaction Master Mix (RM)	10 μl
Nuclease free water	6 μl
Total volume	18 μl

Note: Use a new tip when pipetting QD / RM / Nuclease free water.
Note: Thaw the reagents and mix thoroughly before use.

5.4 Reaction setup

- Use the appropriate reaction tubes or 96-well reaction plate for the QPCR reaction.
- Transfer 18 μl of premix solution into each well then dispense 2 μl of diluted unknown sample or standard into each well.
- Cap the reaction tubes or seal the PCR plate, then briefly spin down the reaction mix.

6. QPCR Protocol

6.1 Detection channels

Assign the corresponding detector as listed in the following table:

Detection	Reporter
WSSV genome	FAM
Shrimp genome	VIC

6.2 Assigning the Information of Plate Positions

Assign both of the two detectors to the wells containing reactions and enter the sample names of each well. Then select the corresponding function (Task) for each sample type according to the following table:

Sample Type	Function (Task)	Concentration (Quantity)
Sample	Unknown	-
Non-template control	NTC	-
Standard	Standard	10^6-10^2

To generate a standard curve, use all WSSV QD Standards (106-102) per PCR run and enter the corresponding concentrations for each standard.

Please note that you can use ROX as a passive reference to normalize tube-to-tube variations if your equipment has the ROX calibration function.

6.3 QPCR Program

Use these temperature settings and cycles. Make sure the reaction volume is set to 20 µl.

Temperature	Time	Cycles
50℃	2 mins	1
95℃	10 mins	1
95℃	15 secs	40
60℃	1 mins	

7. Data Analysis

7.1 Software Settings

Use the default settings in your software.

7.2 Check Shrimp DNA signal

If shrimp signal (VIC) is not detected, no diagnosis can be concluded. It may be caused by reaction failure or PCR inhibition.

7.3 Check WSSV DNA signal

If shrimp signal (VIC) is detected and at the same time WSSV genome fluorescence signal is not detected or under limit of quantification (< 10 copies), then the sample can be considered negative.

7.4 Quantitation of WSSV

If both WSSV and Shrimp DNA signals are detected, the sample is considered positive, and it means the sample contains WSSV DNA.

To get the results (copies/μl), the following equation must be applied to convert the values into WSSV copies per nanogram of shrimp DNA:

$$\text{Shrimp DNA (ng/μl)} = \frac{\text{Shrimp DNA copy number (copies/μl)}}{\text{Shrimp index (copies/ng)}}$$

$$\text{Ratio of virus copy to shrimp DNA} = \frac{\text{WSSV DNA copy number (copies/μl)}}{\text{Shrimp DNA (ng/μl)}}$$

The shrimp index may vary from different shrimp species because of different genome size. The shrimp index of white shrimp is 10872 and the index of tiger prawn is 17242. When testing other species, the index should be recalculated.

7.5 Flow Chart of Diagnosis