Pseudocapillaria tomentosa REAL-TIME PCR KIT INSTRUCTIONS

INTRODUCTION

Pseudocapillaria tomentosa is a species of parasitic nematode (roundworm) that primarily infects freshwater fish, particularly zebrafish (Danio rerio) and other small aquarium or laboratory species. It is found primarily in the intestinal tract. Symptoms of infection include weight loss and emaciation, lethargy and reduced feeding, pale coloration, and stringy feces. In severe cases, intestinal inflammation, necrosis and death can occur. *P. tomentosa* is highly contagious within recirculating systems or shared water environments. Its eggs are highly resilient, and can survive for weeks in tank debris, filters, or biofilms. Low-level infections can persist undetected for months, slowly spreading through a colony. Zebrafish facilities infected with *P. tomentosa* may face research interruptions, animal use suspensions, and costs associated with depopulation, sanitation, and repopulation. PCR testing is the best and most sensitive method for detecting its presence in aquatic animal colonies.

PRINCIPLE OF THE TEST

This VRL kit is designed for specific *in vitro* detection of the *Pseudocapillaria tomentosa* genome. The reaction contains a hydrolysis (or TaqMan®) probe that supports quantitative analysis via TEX615 fluorescence.

The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with 100% of the NCBI database reference sequences available at the time the test was designed.

NOTE: FOR RESEARCH USE ONLY

HANDLING

- Each kit contains enough reagents to perform 200 tests.
- After receiving the kit, please store different components of the kit at the proper temperatures.
- All reagents can be used before the expiration date printed on the box.
- PCR reagents should be aliquoted when necessary to avoid more than 2 freezing and thawing cycles.

MATERIALS PROVIDED

Kit Components	Amount	Storage Temp.
P. tomentosa primer/probe mix*	2 vials	Dry: 4 ℃
		After reconstitution: -20 ℃
P. tomentosa 10X positive control	2 vials	Dry: 4 ℃
		After reconstitution: -20 ℃
RNase/DNase free water	1 mL	-20 ℃ to Ambient
2X qPCR Reaction Mix	2 x 1.1 mL	-20 ℃
TE Buffer	1 mL	-20 ℃ to Ambient

*: Light sensitive: Protect from light

REQUIRED BUT NOT PROVIDED

1. Extraction Kits (see details below)
2. Real-time PCR instrument.
3. Microcentrifuge
4. Pipettes (0.5 µL - 1 mL) with sterile filter tips
5. 1.5 - 2 ml sterile micro centrifuge tubes
6. 96-well, thin wall PCR plates
7. Individual PCR tubes, 8-tube strip
8. Powder-free gloves

WARNINGS AND PRECAUTIONS

- This test must be carried out by skilled personnel.
- Strictly divide sample and reagent preparation areas physically from the amplification room/area.
- Samples should be regarded as potentially infectious materials.
- Store positive and/or potentially positive materials separated from all other components of the kit.
- Do not use the kit after its expiration date.
- Always use sterile filter tips for pipetting.
- Pipettes, vials and other working materials should not circulate among separate workspaces.
- Wear separate coats and gloves in each area.
- Routinely decontaminate your pipettes and benches with disinfectants such as bleach, DNA AWAY™, or RNase AWAY™.

➢ DNA extraction

1. The following kits are recommended for preparation of extracted DNA for this test. However, any process that yields high quality DNA with minimal PCR inhibitors is acceptable:

   Kit for DNA extraction from whole fish samples: Qiagen QIAamp Fast DNA Stool Mini Kit (Qiagen #51604), in conjunction with a tissue grinder.

   Kit for DNA extraction from environmental samples: Qiagen QIAamp DNA Mini Kit (Qiagen #51306).

2. **It is recommended to store extracted DNA at -20℃.**

➢ P. tomentosa Real-time PCR

■ Reagent Resuspension

1. To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Filtered tips are recommended for all pipetting steps.
2. Pulse-spin each tube in a microcentrifuge before opening.
3. Reconstitute the kit components in the RNase/DNase-free water supplied, according to the following table:

Component	Volume water
P. tomentosa primer/probe mix*	50 µL (each vial)
10X P. tomentosa positive control	80 µL (each vial)

*: Light sensitive, please protect from light

4. To ensure complete resuspension, vortex each tube thoroughly.
5. Resuspend the primer/probe mix vials and positive control vials sequentially, only as needed.
6. Note: The positive control contains high-copy number template and is a significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the pre-PCR components (i.e., primer/probe mix).

■ qPCR setup protocol

1. For each extracted sample, prepare a reaction mixture according to the table below. Include sufficient reactions for positive and negative controls:

   Per reaction

Component	Volume
P. tomentosa primer/probe mix	0.4 µL
2X qPCR Reaction Mix	10 µL
RNase/DNase Free water	4.6 µL
FINAL VOLUME	**15 µL**

2. Pipette 15 µL of reaction mix into each well/plate according to your qPCR experimental plate

setup.

3. Pipette 5 µL of DNA-extracted sample into each well, according to your experimental plate setup.
4. Preparation of 1X positive control: Dilute 0.8 µL 10X positive control with 7.2 µL TE buffer. Load 5 µL into the appropriate well.
5. For blank control wells, use 5 µL RNase/DNase free water.
6. The final volume in each well must be 20 µL.
7. If a standard curve is to be included for quantitative analysis, prepare a standard curve dilution series:
    a. Pipette 18 µL TE buffer into 4 tubes and label tubes 1-4.
    b. Pipette 2 µL 10X positive control into tube 1.
    c. Vortex thoroughly, then briefly spin down the contents.
    d. Change pipette tip and pipette 2 µL from tube 1 into tube 2.
    e. Vortex thoroughly, then briefly spin down the contents.
    f. Repeat steps d. and e. to complete the dilution series

Standard Curve	Copy Number
10X Positive control	$1x10^4$ per reaction
Tube 1	$1x10^3$ per reaction
Tube 2	$1x10^2$ per reaction
Tube 3	$1x10^1$ per reaction
Tube 4	$1x10^0$ per reaction

    g. Pipette 5 µL of standard template into each well for the standard curve according to your experimental plan setup.

■ qPCR setup suggestions

8. Prepare and aliquot primer/probe mix inside a biosafety cabinet (BSC) or PCR workstation in a pre-PCR room/area. That is, separate from where samples, PCR positive controls are handled, and separate from the amplification room. Keep all reagents cool (or on ice) at each experimental step.
9. In the pre-PCR room/area, prepare and mix the reaction mixture thoroughly, then pipette 15 µL into each reaction tube or well of a 96-well plate.
10. Transfer the reaction tubes or plate into the PCR assembly or amplification room. In a BSC or PCR workstation add the blank control, extracted DNA sample, and positive control to the reaction mix. Use tips with aerosol filters to add sample and positive controls
11. Add 5 µL in the following order: Blank control ultrapure water, sample DNA, and positive control(s) into the designated tube/well, mix carefully by pipetting.
12. Centrifuge tubes briefly to spin down the contents.

■ qPCR run protocol

	Step	Temp	Time	Cycles
1	Initial Denaturation	95 °C	3 min	1
2	Denaturation	95 °C	15 sec	45
	Annealing/Extension	60 °C	60 sec	

Fluorogenic data should be collected through a channel compatible with TEX615 fluorescence (i.e. red fluorescent channel; Texas Red or rhodamine channel)

1. For basic information regarding the setup and programming of your real-time PCR instrument, please refer to the user manual.
2. Load the tubes/plate in the thermal cycler and start the run.

INTERPRETATION OF RESULTS

The results are valid when the controls and the samples meet all the requirements as follows:

✧ Expected results for controls

Controls	TEX615 channel	
	Cq Value	Amplification curve
Blank Control (BC)	None or >38.0	-
1X Positive Control (PC) ($1x10^3$ copies per reaction)	22 - 30	Typical (S-shaped curve)

Note: If Cq of blank control is ≤38.0, then the test is invalid and must be repeated.

✧ Results for samples

TEX615 Channel		Results
Cq Value	Amplification curve	
≤38.0	S-shape	Positive
None detected	-	Negative
Or >38.0		