

Materials List for:

Detection of Tilapia Lake Virus Using Conventional RT-PCR and SYBR Green RT-qPCR

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Materials

Name	Company	Catalog Number	Comments
Tissue collection			Step 1
Tricaine methanesulfonate	Sigma-Aldrich	E10521	An alternative to clove oil. Step 1.1
RNAlater stabilization solution	Thermo Fisher Scientific	AM7020	For storing tissues if they cannot be processed immediately Step 1.3
RNA extraction			Step 2
TRIreagent	Sigma-Aldrich		Step 2.1
TRIzol	Thermo Fisher Scientific (Invitrogen)	15596026	Step 2.1
GENEzol	Geneaid	GZR100	Step 2.1
Trisure	Bioline	BIO-38032	Step 2.1
Homemade solution	-	-	94.53 g/L (800 mM) guanidine thiocyanate 30.45 g/L (400 mM) ammonium thiocyanate 8.20 g/L (100 mM) sodium acetate 380 mL/L (38 % v/v) phenol 50 mL/L (5 % v/v) glycerol 1.0 g/L (0.1 % w/v) 8-quinolinol, pH 5.0 Store up to 2 years at 4°C Step 2.1
MagNA Lyser Green Beads	Roche	3358941001	An alternative tissue homogenization method used in conjunction with tissue lysing machines detailed below Step 2.2
Lysing Matrix D, 2 mL Tube	MP BIOMEDICALS	116913050	
Chloroform	Sigma-Aldrich	C2432	Step 2.3
Chloroform	RCI Labscan	AR1027E-G2.5L	Step 2.3
1-Bromo-3-chloropropane	Sigma-Aldrich	B9673	A less toxic alternative to chloroform Step 2.3
Isopropanol (GC) ≥ 99.8 %	Sigma-Aldrich	59300	Step 2.6
Isopropanol (ACS, ISO Reag. Ph Eur)	Merck KGaA (EMSURE)	1.09634.2500	Step 2.6
Glycogen, molecular biology grade (e.g., Sigma, cat. no. G1767)	Thermo Fisher Scientific (Thermo Scientific)	R0551	Useful step if tissue starting material is small to maximise RNA precipitation

			optional
Ethanol (purity (GC) ≥ 99.9 %)	Sigma-Aldrich (EMD Millipore)	1.00983	Step 2.9
Ethanol (ACS, ISO Reag. Ph Eur)	Merck (EMSURE)	1.00983.2500	Step 2.9
Nuclease-free water	Promega	P1193	Step 2.13
Nuclease-free water	Multicell	809-115-CL	Step 2.13
Ambion TURBO DNA-free kit	Thermo Fisher Scientific (Invitrogen)	AM1907	Can be performed at the end of the RNA extraction protocol optional
cDNA synthesis			Step 4
Viva cDNA Synthesis Kit	Vivantis	cDSK01	Step 4.1 & 4.3
ReverTra Ace qPCR RT MasterMix with gDNA remover	Toyobo	A1172K	An alternative option see discussion
ReverTra Ace qPCR RT Kit	Toyobo	FSQ-101	An alternative option see discussion
AffinityScript Multiple Temperature Reverse Transcriptase	Agilent Technologies	600107	An alternative option
PCR			Step 5
DNA polymerase systems:			Step 5.2
- Platinum II Hot-Start Green PCR Master Mix (2X)	Thermo Fisher Scientific (Invitrogen)	14001012a	Step 5.2
- GoTaq Mastermix	Promega	M7122	Step 5.2
Separate PCR mixture components:			Step 5.2
10mM dNTP Mix	Vivantis	NP2409	Step 5.2
25mM MgCl ₂	Thermo Fisher Scientific	R0971	Step 5.2
10X Taq Buffer with KCl	Thermo Fisher Scientific	00348114	Step 5.2
Taq DNA polymerase	Vivantis	PL1202	Step 5.2
- Verso 1-step RT-PCR ReddyMix with ThermoPrime Taq	Thermo Fisher Scientific	AB1454	One step RT-PCR exemplified in Figure 3B
Gel electrophoresis:			For visualization of PCR products from steps 5.1-5.4
Ethidium Bromide solution (10 mg/mL)	Thermo Fisher Scientific	17898	Step 5.5
Tris/Acetic/EDTA (TAE) buffer:			Step 5.5
- Tris	Vivantis	PR0612-1KG	Step 5.5
- Acetic acid (glacial) (ACS, ISO, Reag. Ph Eur)	Merck KGaA (EMSURE)	1.00063.2500	Step 5.5
- Ethylenediaminetetraacetic acid (EDTA)	BIO-RAD	161-0729	Step 5.5
Agarose	Vivantis	PC0701-100G	Step 5.5
DNA ladders and markers	Vivantis	NL1405	Step 5.5
DNA gel loading dye (6X)	Thermo Fisher Scientific	R0611	Step 5.5
qPCR			Step 6
PowerUP SYBR Green Master Mix	Thermo Fisher Scientific (Applied Biosystems)	A25779	Exemplified in Figures 4-6B Step 6.2
iTaq Universal SYBR Green Supermix	BIO-RAD	1725120	Exemplified in the video and in Figures 4-6A Step 6.2
Equipment			
Dounce tissue grinder pestle	Sigma-Aldrich	P1110	Protocol 2

MagNA Lyser Instrument	Roche	3358976001	An alternative tissue homogenizing option for protocol 2 which are used in conjunction with the lysing beads detailed above Step 2.2
FastPrep-24 5G Homogenizer	MP BIOMEDICALS	116005500	
Refrigerated microcentrifuge	Eppendorf	Eppendorf 5427R	Protocol 2 Step 2.4, 2.7 & 2.10
Refrigerated microcentrifuge	Eppendorf	Eppendorf 5418R	
Heat box	Labnet	AccuBlock Digital Dry Bath	Protocol 2 Step 2.13
Microvolume spectrophotometer	Thermo Fisher Scientific (Applied Biosystems)	Nanodrop 2000	Protocol 3 Step 3.1 - 3.4
PCR machine	BIO-RAD	T100 Thermal Cycler	Protocol 5 Step 5.4
Power supply	BIO-RAD	PowerPac HC	Protocol 5 Step 5.5
Horizontal gel electrophoresis	BIO-RAD	Mini ReadySub-Cell GT Cell #1704487edu	Protocol 5 Step 5.5
Mini microcentrifuge	Corning	LSE 6766	Useful to quickly spin down PCR reaction tubes in protocols 4, 5 & 6 Step 6.5.1
Microcentrifuge	LioFuge	LM-60	Step 6.5.1
qPCR machine and software	Thermo Fisher Scientific	7500 Fast Real-Time PCR System with 7500 Software v2.0	Protocol 6 Step 6.6-6.8
qPCR machine and software	BIO-RAD	CFX96 Touch Real-Time PCR Detection System with CFX Manager software	
General Materials			
Mayo scissors			Step 1.1-1.2
Forceps			Step 1.1-1.2
Pipette	Rainin	Pipette-Lite XLS	
Aerosol-barrier pipette tips	Sigma-Aldrich	Z333328, Z333336, Z333344	
Nuclease-free 1.5-ml microcentrifuge tubes	Eppendorf		