# Chapter 3-XIV Total RNA Extraction from Fish and Clam



# **Total RNA Extraction from Body Cavity Fluid of Fish**

#### Protocol

1.5 ml Eppen tube - Body cavity fluid of fish: 100 µl \*1 LRT mix and 3 µl of 2-ME : 300 µl \*2 Vortexing (maximum speed): 30 sec Flash spin down Vortexing (maximum speed): 30 sec Keep 5 min at room temp Flash spin down → >99% ethanol : 200 µl Vortexing (maximum speed): 30 sec Flash spin down Lysate Transfer all contents of the micro tube into the cartridge of QuickGene Refer to the extraction protocol of the device used. QuickGene-810 to p.4-8 QuickGene-Mini80 to p.4-15 QuickGene SP kit to p.4-22 Total RNA

- \*1 In case freezing and melting was repeated, centrifuge at 6,800×g for 3 min and collect supernatant. \*2 LRT mix: Dissolve 310 mg of Carrier RNA with 11.6 ml of LRT

#### Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination: A260/280

Amount of body cavity fluid	A260/280
100 μΙ	1.6

Chaotropic salt contamination: A260/230

No Data

Other

No Data

# Common protocol is usable for the following

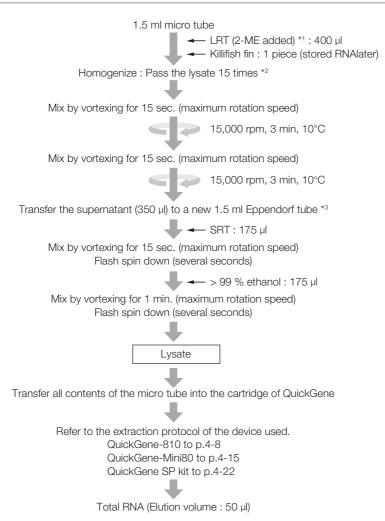
No Data





# **Total RNA Extraction from Fin of Killifish**

### Protocol



\*1 Add 10 µl of 2-ME per 1 ml of LRT.

\*2 Until particles of tissue becomes invisible

\*3 Take care not to suck the precipitate

#### Results

Electropherogram

No Data

The yield of total RNA

Amount of fin	Yield(µg)
1 piece	2.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

# Common protocol is usable for the following

Killifish Liver





#### RD-3

# **Total RNA Extraction from Liver of Killifish**

#### Protocol



← LRT (2-ME added) \*1 : 400 μI

Killifish liver : 1 piece (stored RNAlater)

Homogenize: Pass the lysate 15 times \*2

Mix by vortexing for 15 sec. (maximum rotation speed)

15,000 rpm, 3 min, 10°C

Mix by vortexing for 15 sec. (maximum rotation speed)

15,000 rpm, 3 min, 10°C

Transfer the supernatant (350  $\mu$ l) to a new 1.5 ml Eppendorf tube  $^{*3}$ 

**→** SRT : 175 μl

Mix by vortexing for 15 sec. (maximum rotation speed) Flash spin down (several seconds)

→ > 99 % ethanol : 175 µl

Mix by vortexing for 1 min. (maximum rotation speed) Flash spin down (several seconds)



Transfer all contents of the micro tube into the cartridge of QuickGene



Refer to the extraction protocol of the device used.

QuickGene-810 to p.4-8

QuickGene-Mini80 to p.4-15 QuickGene SP kit to p.4-22

Total RNA (Elution volume : 50 µl)

\*1 Add 10 µl of 2-ME per 1 ml of LRT.

\*2 Until particles of tissue becomes invisible

\*3 Take care not to suck the precipitate

#### Results

Electropherogram

No Data

The yield of total RNA

Amount of liver	Yield(µg)
1 piece	about 20.0

Protein contamination: A260/280

Amount of liver	A260/280
1 piece	2.1

Chaotropic salt contamination : A260/230

No Data

Other

No Data

# Common protocol is usable for the following

Killifish fin

