14. Total RNA Extraction from Fish and Clam

RD-1

Total RNA Extraction from Body Cavity Fluid of Fish

Protocol

1.5 ml micro tube

↓ ← Body cavity fluid of fish : 100 µl *1

↓ ← LRT mix and 3 µl of 2-ME : 300 µl *2

Vortex (maximum speed)：30 sec

Flash spin down

↓ ← SRT : 200 µl

Vortexing (maximum speed) : 30 sec

Flash spin down

↓

Incubation at RT：5 min

↓ ← >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 for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

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

Protein contamination：A260/280

Amount of body cavity fluid	A260/280
100 µl	1.6

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RD-2

Total RNA Extraction from Fin of Killifish

Protocol

1.5 ml micro tube

↓ ← LRT (2-ME added) *1 : 400 µl

↓ ← Killifish fin : 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 µl) to a new 1.5 ml micro tube *3

↓ ← SRT : 175 µl

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

↓ ← >99% ethanol : 175 µl

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

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

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

The yield of Total RNA

Amount of fin	Yield (µg)
1 piece	2.0

Common protocol is usable for the following

Killifish liver

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RD-3

Total RNA Extraction from Liver of Killifish

Protocol

1.5 ml micro tube

↓ ← LRT (2-ME added) *1 : 400 µl

↓ ← 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 µl) to a new 1.5 ml micro tube *3

↓ ← SRT : 175 µl

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

↓ ← >99% ethanol : 175 µl

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

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

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

The yield of Total RNA / Protein contamination：A260/280

Amount of fin	Yield (µg)	A260/A280
1 piece	2.0	2.1

Common protocol is usable for the following

Killifish fin

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).