Total RNA extraction from Common carp tissue (BodyMap)

Caution!

When using RNA extraction kits:

- check the safety data sheets for RNA extraction kit contents
- always wear a lab coat, gloves and eye protection
- perform all steps of the procedure under the fume hood
- don't mix bleach with RNA extraction sample-preparation waste – contains a guanidine salt, which can form hazardous compounds when combined with bleach – if it spills clean the area first with laboratory detergent and water

Reagents, equipment and supplies

- miRNeasy Mini Kit (Qiagen, 217004)
- 2 mL tubes with 3 mm steel beads (Macherey-Nagel, 740814.50)
- 70% and 96-100% ethanol
- chloroform
- nuclease-free 1.5 ml eppendorf tubes
- microcentrifuge for 1.5 ml eppendorf tubes
- shaker for 1.5 ml eppendorf tubes
- ice for defrosting RNA samples

Before start

- perform all steps at room temperature except phase separation step (centrifugation at 4°C) - pre-cool microcentrifuge to 4°C
- before first use add the indicated volume of 96-100% ethanol to RWT and RPE buffer concentrates
- avoid ribonuclease contamination (wear gloves when handling RNA samples and reagents and change them frequently; use sterile, RNase-free pipette tips and appropriate reagents to remove RNase contamination from work surfaces and non-disposable elements; close bottles with kit components immediately after use and keep them tightly sealed when you are not using them)

Procedure

1. If samples are frozen in RNA Later let them thaw keeping them on ice.
2. Add 700 ul QIAzol Lysis Reagent to each prepared tube with steel beads.
3. Transfer the tissue (up to 30 mg, cut the tough-to-lyse tissue i.e. gill or muscle into smaller pieces) to the tube with a previously added QIAzol Lysis Reagent and homogenize with maximum speed using shaker for 1.5 ml eppendorf tubes until a complete disruption of tissue.
4. Incubate the homogenate at room temperature for 5 minutes.
5. Transfer the lysate to a new labeled nuclease-free 1.5 ml eppendorf tube and add 140 ul chloroform. Cap tube securely and shake vigorously for 15 seconds.
6. Incubate at room temperature for 2–3 minutes and then centrifuge for 15 minutes at 12000 x *g* at 4°C.
7. Transfer the upper aqueous phase to a new labeled nuclease-free 1.5 ml eppendorf tube, avoiding transferring any interphase. Add 525 ul of 100% ethanol, and mix by pipetting.
8. Place a RNeasy Mini column in a 2 ml collection tube.
9. Pipet up to 700 ul sample, including any precipitate, into an RNeasy Mini column, close the lid and centrifuge at 8000 x *g* for 15 seconds at room temperature. Discard the flow-through. Repeat this step using the remainder of the sample.
10. Add 700 ul RWT Buffer to the RNeasy Mini column, close the lid and centrifuge for 15 seconds at 8000 x *g*. Discard the flow-through.
11. Add 500 ul RPE Buffer to the RNeasy Mini column, close the lid and centrifuge for 15 seconds at 8000 x *g*. Discard the flow-through.
12. Add 500 ul RPE Buffer to the RNeasy Mini column, close the lid and centrifuge for 2 minutes at 8000 x *g*. Discard the flow-through.
13. Place the RNeasy Mini column into a new 2 ml collection tube and centrifuge at full speed for 1 minute.
14. Transfer the RNeasy Mini column to a new labeled nuclease-free 1.5 ml eppendorf tube, add 30-50 ul of RNase free water directly onto the RNeasy Mini column membrane. Close the lid and centrifuge for 1 minute at 8000 x *g* to elute.
15. The eluted RNA can be used immediately or stored frozen.