RNA extraction from Atlantic Salmon head kidney tissue

Product	Company	Reference
Water Molecular Biology Grade (1000ml)	Thermo Scientific	SH30538.03
Tungsten Carbide Beads	Qiagen	69997
RNase*Zap*®, 250 mL, s, cat. no. , £47.20	Life technologies	AM9780
Sterile Microtubes	Axygen	MCT-150-C-S
TRI Reagent®	Sigma	T9424-200ML
RNAlater®	sigma	R0901-500ML
Chloroform	sigma	C2432-1L
2-Propanol	sigma	I9516-500ML
Molecular grade 100% Ethanol	sigma	2483

- Thaw head kidney in RNAlater kept at -80°C
- Take a small piece of the tissue (10-20mg), get rid of excess RNAlater using a clean paper towel.
- Add the tissue into 1.5 mL of cold TRIzol in a 2 mL tubes with 2 Tungsten (3 mm) beads
- Homogenise at frequency 30.0 l/sec for 2.5 min, 30 sec breaks
- Incubate on ice for 5 minutes to permit complete dissociation of the nucleoproteins complex
- Add 0.3ml of chloroform, vertex to mix well
- Incubate for 5 minutes on ice
- Centrifuge the sample for 15 minutes at 12,000xg at 4°C
  - The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase
- Transfer the aqueous phase containing the RNA to a new tube, be careful to not take any of the interphase layer
- Add 0.7ml of isopropanol to the aqueous phase, mix well by vertexing
- Incubate for 10 minutes and centrifuge for 10 minutes at 12000xg at 4°C
  - Total RNA precipitate forms a white gel-like pellet at the bottom of the tube
- Discard the supernatant
- Resuspend the pellet in 1ml of 80% ethanol per 1ml of trizol used for lysis, vertex until pellet detached
- Centrifuge for 5 minutes at 7500xg at 4°C
- Discard the supernatant and repeat the washing with 80% ethanol two more times

- Pour away alcohol (watch the pellet), then use 20 µL pipette to remove alcohol (do not touch the pellet), close tubes & spin down till 3500xg. Use 10 µL tips to get rest of alcohol (do not touch the pellet), close the tubes, spin down till 3500xg, then use 10 µL tips again for the final alcohol removal (make sure that all alcohol is gone, otherwise the alcohol in the sample may inhibit qPCR), close tubes.
- Let the RNA pellet air dry for 10 minutes on ice, or if needed, put tubes on hot plate 65°C for 1 min to make sure all alcohol evaporates
- Dissolve the pellet in 100ul of nuclease free water
- Flip the tube with the finger and leave the tubes for 30 min on wet ice
- If pellet is not dissolved after 30 minutes of incubation, incubate the tubes at 65C on hot plate for 10 minutes to properly dissolve the pellet
- Transfer the RNA solution into a new RNAase free tube and check quantity and quality on nanodrop and bioanalyzer
- Store on -80C for longer storage