Total RNA extraction from Atlantic salmon Head Kidney Leukocytes

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

RNA extraction from 6/12 cell plates

- Centrifuge the plate at 500g for 5 minutes to make sure that cells are stuck on the bottom
- Put sterile papers on the sink and quickly invert the plate emptying the growth media without loosing cells. Make sure you have removed all the media.
- In cell culture hood, add trizol depending on the cell count. Add 0.5ml of trizol into each well if you 1-2million cell count
- Pipette up and down all over the well to get all the cells attached to the bottom, put trizol and cell mixture in to a 1.5/2ml Eppendorf tube

You can freeze the samples at this point for later extraction, otherwise proceed with the protocol

- Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex
- Add 0.2ml of chloroform per 1ml of trizol used for lysis, vertex to mix well
- Incubate for 2-3 minutes
- Centrifuge the sample for 15 minutes at 12,000xg at 4C
  - 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 0.5ml of isopropanol to the aqueous phase, per 1ml of trizol used for lysis, mix well by vertexing
- Incubate for 10 minutes and centrifuge for 10 minutes at 12000xg at 4C
  - 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 4C
- 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 40ul 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
- Store on -80C for longer storage