Promega

Product Application

Salmon Tissue RNA Purification

Manual purification of RNA from salmon tissue using the ReliaPrep™ RNA Tissue Miniprep System in ~30 minutes

Kit: ReliaPrep™ RNA Tissue Miniprep System (Cat. #Z6110)

Analyses: UV absorbance and QuantiFluor® quantitation, Bioanalyzer, qPCR amplification, gel electrophoresis

Sample Type(s): Chinook salmon (*Oncorhynchus tshawytscha*) preserved in RNA*later* (Brain, Heart, Kidney, Liver, Spleen and Gill)

Input: 20mg (500µl) of homogenized tissue

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM394, available at: www.promega.com/protocols or contact **techserv@promega.com**

Materials Required:

- ReliaPrep™ RNA Tissue Miniprep System (Cat. #Z6110)
- RNA*later* (Life Technologies Cat. #AM7021)
- Tissue Tearor (BioSpec) or other tissue homogenizer

Protocol

1. Remove tissue samples from the fish, cut into small pieces (~0.5cm) and immediately place in vials with RNA*later*.
2. Store samples for 24 hours in RNA*later* at 4°C.
3. Remove the RNA*later* from the vials and place the samples at -70°C for long term storage.
4. Homogenize tissue samples using the Tissue Tearor in Lysis Buffer (LBA) + 1-Thioglycerol at a final concentration of 40mg/ml.
5. Transfer 500µl of homogenate to a clean microtube.
6. Pipette homogenate 7–10 times to shear the DNA using a P200 or P1000 pipettor.
7. Proceed with step 7 based on fibrous or non-fibrous tissue sample.
    a. For fibrous tissues (heart and gill), add an equal volume (500µl) of RNA Dilution Buffer (RDB) and mix by vortexing for 10 seconds. Incubate 1 minute at room temperature. Clear homogenates by centrifugation at room temperature for 3 minutes at 10,000 × *g* to pellet insoluble debris.
    b. For non-fibrous tissues (kidney, liver, spleen, brain), clear homogenates by centrifugation for 3 minutes at 14,000 × *g*.
8. Transfer cleared lysates to clean tubes, taking care to avoid any pelleted debris.
9. Add 170µl of 100% isopropanol to sample.
10. Transfer the lysate to a ReliaPrep™ Minicolumn.

Proceed with the protocol in the technical manual (TM394) to purify the RNA using the ReliaPrep™ minicolumn.

Results:

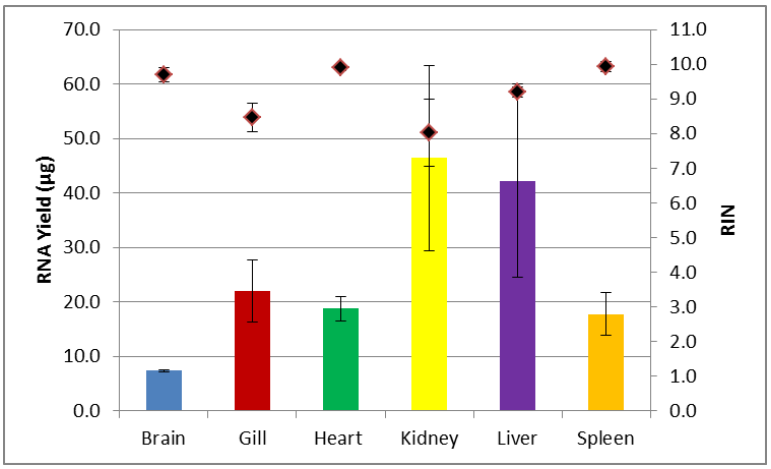

Figure 1. RNA quality - yields and RIN values from salmon tissues. RNA from each tissue type (N= 3 or 4) was analyzed using the NanoDrop 1000 spectrophotometer. >7ug of RNA was recovered from brain, >17ug of RNA was recovered from gill, heart, and spleen, while > 40ug of RNA was recovered from kidney and liver tissue. All samples were of high integrity with average RIN values ≥ 8.0.

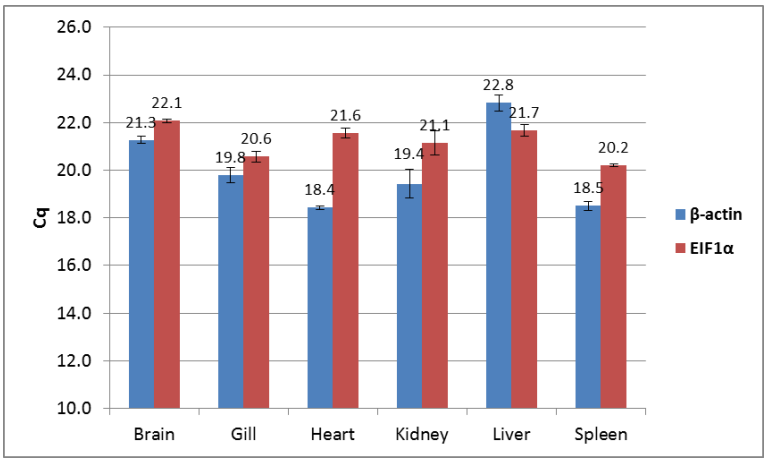

Figure 2. RT-qPCR of RNA from salmon tissues. RNA was analyzed by RT-qPCR using the GoTaq® 1-Step RT-qPCR System on the BioRad CFX96 Real-Time PCR Detection System. Salmon specific primers targeting β-actin and EIF1α transcripts were used in a 20ul total reaction volume with 20ng of RNA. The RNA was amplifiable.

Figure 3. Integrity of RNA from salmon tissues. RNA integrity was determined using the Agilent 2100 Bioanalyzer. The purified RNA was consistently of high integrity with average RIN values ≥ 8.0 across all salmon tissue types tested.