Promega

Product Application

Total RNA Purification from Bone with Cartilage Samples using the Maxwell® RSC Instrument

Purify total RNA including miRNA from bone with cartilage using the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)

Analyses:

- UV absorbance
- Dye-based quantitation
- RT-qPCR
- TapeStation

Sample Type(s): Bone with cartilage sample

Input: 20mg

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Bioreba universal bag (Bioreba, Cat.# 430100)
- Hammer
- Lysing Matrix S (MP Biomedicals, Cat.# 6925100)
- FastPrep-24™ Sample Preparation System (MP Biomedicals, Cat.# 116004500)
- Rotator mixer (optional)

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.

For further information, see Technical Manual TM441, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Thaw the samples and keep them on ice during all steps. Weigh 20mg of sample.
2. Transfer the sample into a plastic bag (e.g., Bioreba universal bag).
3. Homogenize the sample using a hammer.
4. Add 200µl Homogenization Solution + 1-Thioglycerol (previously prepared and pre-chilled).
5. Collect and transfer the lysate (including all solid material) into a Lysing Matrix S tube.
6. Transfer the Lysing Matrix S tube into the FastPrep-24™. Set the FastPrep-24™ at 5m/s for 15 seconds. Bead beat the samples 3 times, keeping the samples on ice for 20 seconds between each repetition.
7. Follow the protocol provided in the Maxwell® RSC miRNA Tissue Kit Technical Manual (TM441) starting at Section 4.A.

   A rotator mixer can be used during the 10-minute incubation step to keep sample particles in suspension (optional).
8. Run the Maxwell® RSC miRNA Tissue Kit method on the Maxwell® RSC Instrument.

Results:

RNA was successfully purified from bone with cartilage samples.

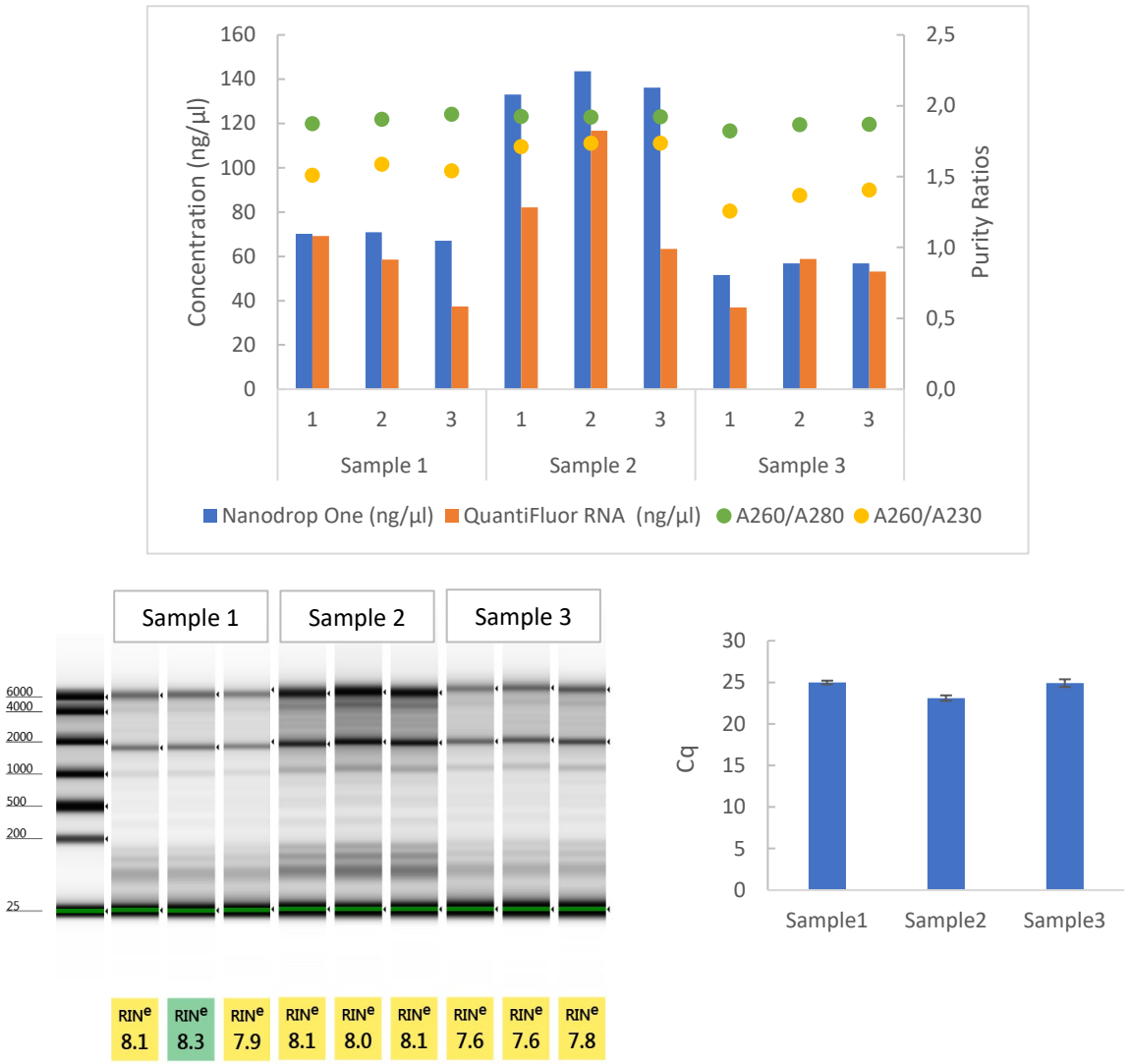

Figure 1. RNA purification from bone with cartilage samples using the Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460) on the Maxwell® RSC Instrument (Cat.# AS4500). 20mg of horse bone with cartilage samples from three sample donors were processed as described in the above protocol. **Top.** Concentration and purity ratios were assessed by NanoDrop™ One and the QuantiFluor® RNA System (Cat.# E3310). Shown are n=3 purification replicates per sample. **Bottom left**. RNA Integrity assessed by 4200 TapeStation using the RNA ScreenTape (Agilent). Shown are n=3 purification replicates per sample**. Bottom right**. RT-qPCR. RNA amplifiability assessed using 2µl of RNA eluates, ACTB[1] primers and the GoTaq® 1-Step RT-qPCR System (Cat.# A6020). Shown is mean ± SD of n=3 replicates, n=1 amplification.

References:

1. Lies Bogaert, *et al.*, (2006) Selection of a set of reliable reference genes for quantitative real-time PCR in normal equine skin and in equine sarcoids, *BMC Biotechnology*. 6:24.