Product Application

Automated RNA Purification from EpiDerm-FT™ Tissue

Purify high quality RNA from an in vitro human model of full thickness skin tissue using the Maxwell® RSC simplyRNA Tissue Kit

Kit: Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340)

Analyses: UV absorbance, dye-based quantitation, Bioanalyzer

Sample Type(s): EFT-412 Skin Model (MatTek Corporation)

Input: 1/4 tissue (~15mg)

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 TM416, available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340)
- RNA*later*®
- RNAseZAP®
- Sterilized Forceps
- Sterilized Dissecting Scissors
- Rotor/stator tissue homogenizer
- 4mm stainless steel beads
- Bead beater homogenizer
- Proteinase K (Cat.# MC5005)

Protocol:

Samples stored in RNA*later*®

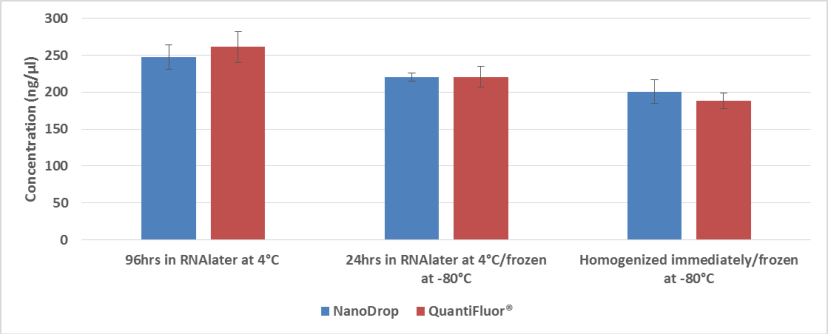
1. Equilibrate EFT-412 tissues in EFT-400 media and prepare according to the manufacturer's recommendations.
2. Remove tissues from the media and rinse in DPBS.
3. Using a sterilized dissecting scissors and forceps pretreated with RNAseZAP®, cut the tissues into quarters and transfer all 4 quarters of the same tissue to a single 2ml tube containing 1.5ml of RNA*later*® and incubate at room temperature for 2 hours.
4. Transfer tube to 4°C.
    a. Keep tube containing four tissue quarters at 4°C for 24 hours. Remove the RNA*later*® and store the tissue at -80°C until RNA purification, or
    b. Keep tube at 4°C until RNA purification.
5. Add one 4mm steel bead and 800µl (200µl x 4) of Homogenization Solution + 1-Thioglycerol to each tube containing the 4 quarter pieces of tissue.
6. Homogenize the sample using a bead beater for 30 seconds at 4m/s followed by cooling on ice for 90 seconds. Repeat for a total of four intervals (2 minutes total homogenization).
7. Centrifuge the sample at maximum speed for 1 minute to reduce foaming.
8. Add 800µl (200µl x 4) of Lysis Buffer to the tube.
9. Add 100µl (25µl x 4) of Proteinase K (20mg/ml) to the tube.
10. Mix well by pipetting.
11. Incubate at room temperature for 10 minutes.

12. Add 425µl of sample (x4) to the Maxwell® RSC simplyRNA Tissue cartridge.
13. Add 5µl of DNaseI to well #4.
14. Add 50µl of Nuclease-Free Water to each elution tube.
15. Place the plunger in the indicated position of the cartridge
16. Select the simplyRNA Tissue method on the Maxwell® RSC. Proceed with the run.

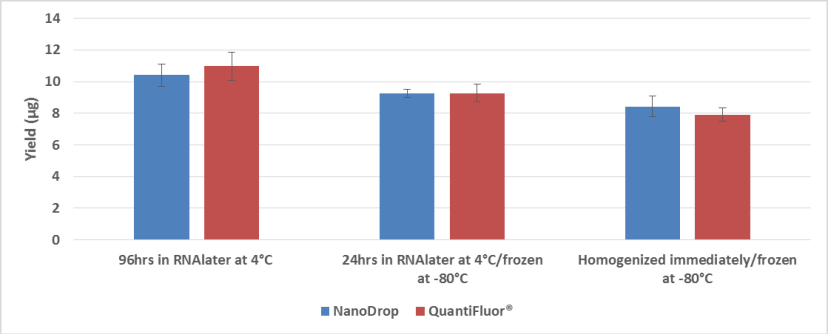
Samples not stored in RNA*later*®

1. Homogenize one entire tissue (4 quarters) using a rotor/stator tissue homogenizer in 800µl (200µl x 4) of Homogenization Solution + 1-Thioglycerol.
2. Store the homogenate at -80°C until RNA purification.
3. Thaw the homogenate on ice and add 800µl (200µl x 4) of Lysis Buffer and 100µl (25µl x 4) of Proteinase K (20mg/ml) to the tube and process as indicated above.

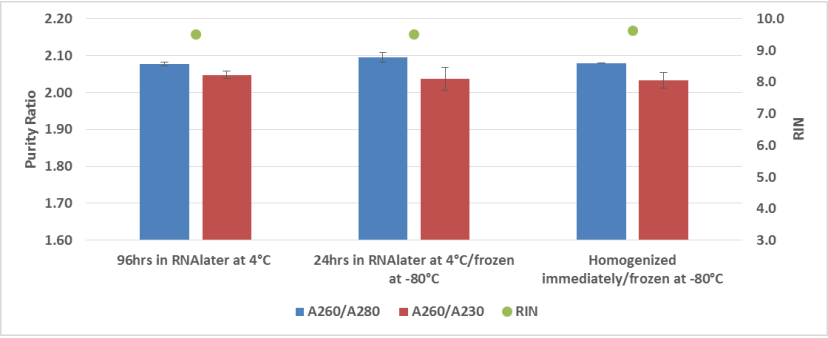
Results:



Concentration of RNA purified from one quarter (~15mg) of EpiDerm-FT™ Tissue. RNA was quantitated using the NanoDrop-1000 and the QuantiFluor® RNA System (Cat.# E3310) on the Quantus™ Fluorometer (Cat.# E6150) (mean ± STD of n=4).



Yield of RNA purified from one quarter (~15mg) of EpiDerm-FT™ Tissue. RNA was quantitated using the NanoDrop-1000 and the QuantiFluor® RNA System (Cat.# E3310) on the Quantus™ Fluorometer (Cat.# E6150) (mean ± STD of n=4).



Purity and integrity of RNA purified from one quarter (~15mg) of EpiDerm-FT™ Tissue. RNA purity was analyzed using the NanoDrop-1000. RNA integrity was analyzed using the Agilent RNA 6000 Nano Kit on the Bioanalyzer (mean ± STD of n=4).