Promega

Product Application

Optimized Mouse Spleen RNA Isolation Protocol

Maximize the quality of RNA isolated from mouse spleen tissue using the Maxwell® simplyRNA Tissue Kit on the Maxwell® RSC Instrument.

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

Analyses: UV absorbance, dye-based quantitation, Bioanalyzer

Sample Type(s): Mouse spleen tissue

Input: Up to 15mg of RNAlater preserved tissue

Materials Required:

- Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340)
- Maxwell® RSC Instrument (Cat.# AS4500)
- RNAlater® Stabilization Solution (ThermoFisher)
- Proteinase K Solution (Cat.# MC5005)
- RNasin® Plus RNase Inhibitor (Cat.# N2611)
- Thermomixer or heat block capable of 56°C
- Hand-held homogenizer

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**

Protocol:

1. Collect mouse spleens directly into RNAlater® and store at room temperature up to one week, 4°C for one month, or -20°C indefinitely.
2. Prior to beginning RNA isolation protocol add 40µl 1-Thioglycerol to each ml of Homogenization Solution (i.e. 600ul in 30ml bottle). For best results prepare fresh but solution can also be stored for up to 30 days at 4°C. Re-suspend DNase I in water and add blue dye as described in TM416.
3. When ready to isolate RNA, remove RNAlater® from tissue.
4. Add 200µl of Homogenization Buffer with 1-Thioglycerol added to up to 15mg of spleen tissue.
5. Homogenize thoroughly on ice with a hand-held homogenizer.
6. Add 35µl of Proteinase K Solution and 2µl of RNasin® Plus RNase Inhibitor and vortex for 10 seconds.
7. Incubate sample at 56°C for 10 minutes.
8. Add 300µl of Lysis Buffer to sample and vortex for 10 seconds.
9. Load sample to well #1 of Maxwell® RSC cartridge.
10. Add 15µl of Re-suspended DNase I into well #4 of Maxwell® RSC Cartridge.
11. Insert plunger and elution tubes filled with 65µl of Nuclease-Free Water into cartridge rack.
12. Run the Maxwell® RSC simplyRNA Tissue protocol on the Maxwell® RSC Instrument.

Results:

Table 1: RNA concentration, yield, and purity ratios isolated from 15mg of mouse spleen tissue using the Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340) on the Maxwell® RSC Instrument (Cat.# AS4500) or by TRIzol®.*Concentration:Yield

Sample	QuantiFluor® RNA*	NanoDrop*	A260/A280	A260/A230
simplyRNA	428 ng/µl : 21µg	399.9 ng/µl : 20µg	2.1	2.2
TRIzol	542 ng/µl : 35µg	662.6 ng/µl : 43µg	1.9	1.9

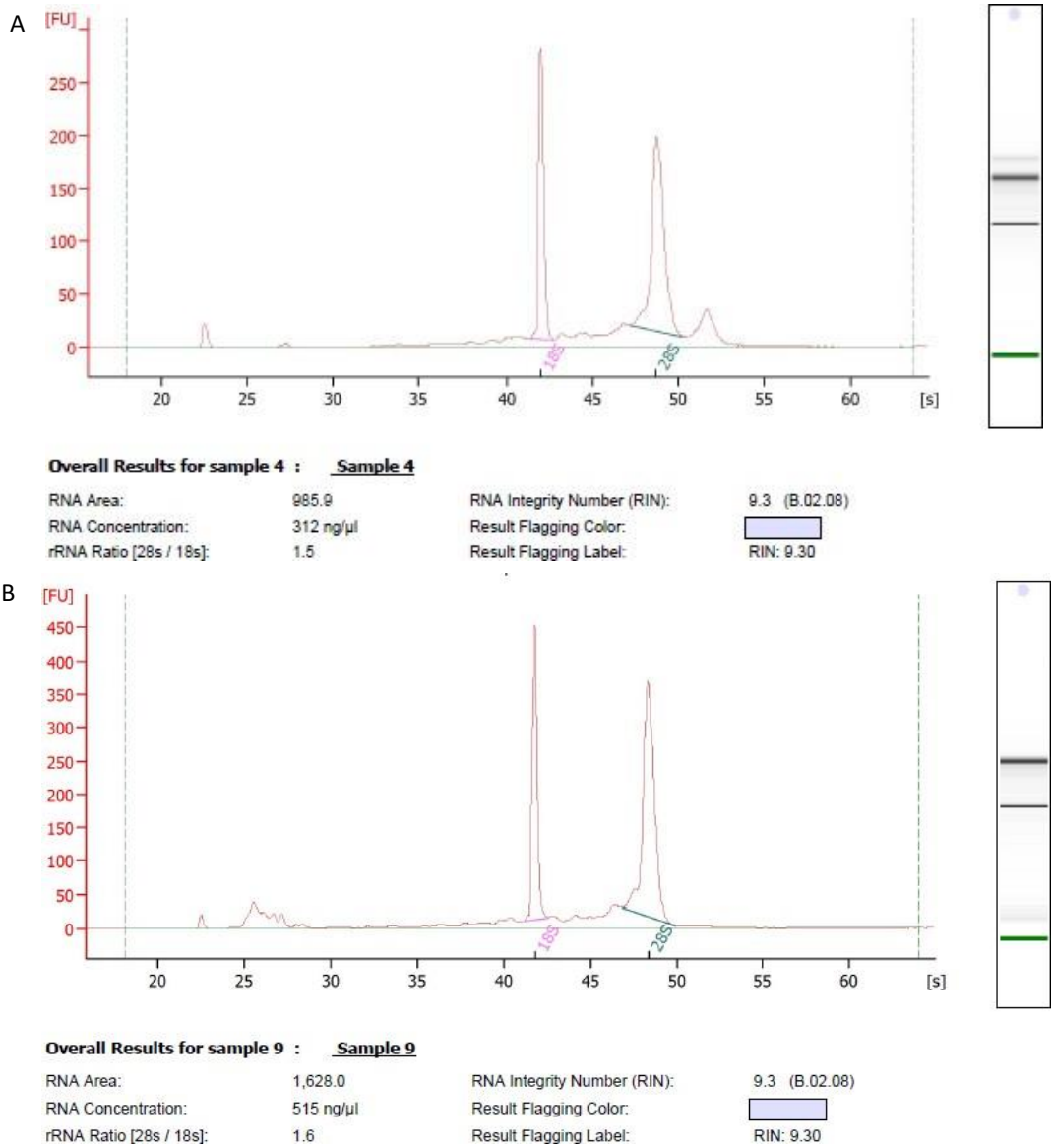

Figure 1. Electropherogram tracing from RNA isolated from mouse spleen tissue using the Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340) on the Maxwell® RSC Instrument (Cat.# AS4500) (Panel A) or by TRIzol® (Panel B). All RNA isolated by the Maxwell® RSC simplyRNA Tissue Kit had RIN scores equal to or greater than 9. RIN and tracings were obtained with the Agilent 6000 RNA Nano Kit (Cat.# 5067-1511)