Promega

Product Application

RNA Purification from Sucrose Gradient Fractions

Purify RNA from a cytosolic cell lysate fractionated with a 15-45% sucrose gradient using the Maxwell® RSC Instrument and Maxwell® RSC miRNA Tissue Kit.

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

Analyses: RT-qPCR, TapeStation

Sample Type(s): 15-45% sucrose gradient fractions

Input: 700µl

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500)

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. Add 200µl of Lysis Buffer, 200µl of Lytic Enhancer and 30µl of Proteinase K to a 700µl sucrose fraction in a 1.5ml tube. Vortex for 20 seconds.
2. Incubate at room temperature for 10 minutes.
3. Meanwhile, prepare the Maxwell® RSC cartridges.
    a. Add 10µl of DNase I to well #4.
    b. Add a plunger to well #8 of each cartridge and Elution Tube to the deck tray.
    c. Add 50µl of Nuclease-Free Water to each Elution Tube.
4. Transfer 1000µl of pre-processed sample to well #1 of the Maxwell® RSC cartridge.
5. Process samples using a Maxwell® RSC Instrument with the miRNA Tissue method.

Results:

RNA was purified from a cytosolic cell lysate fractionated with a 15-45% sucrose gradient using the Maxwell® RSC Instrument and Maxwell® RSC miRNA Tissue Kit. Before RNA purification, each fraction was spiked with 1750pg of Luciferase Control RNA (Cat.# L4561).

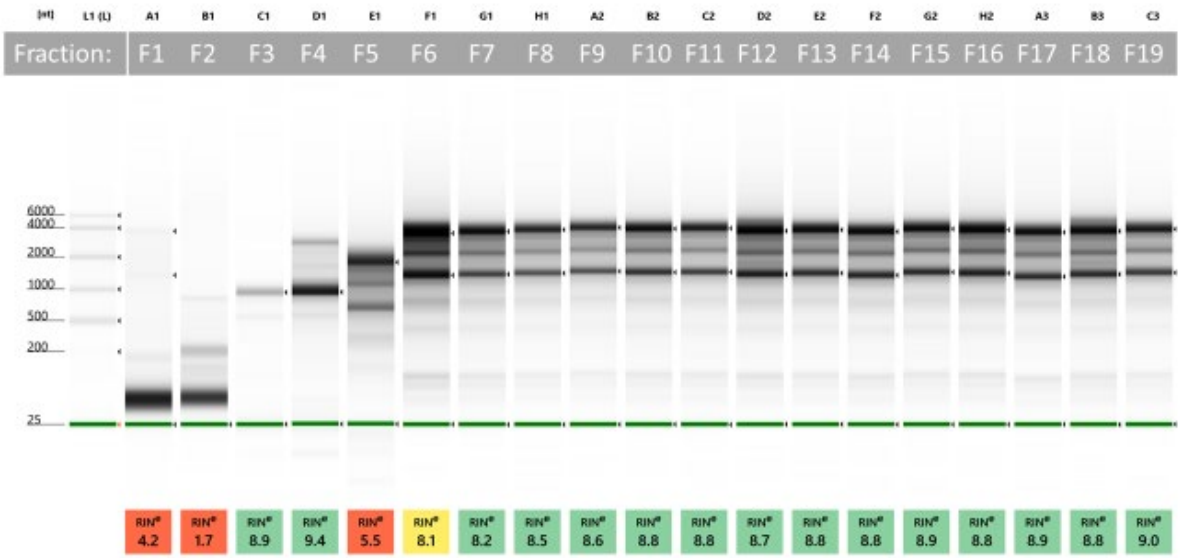

Figure 1. TapeStation electrophoresis of RNA extracted from 15-45% sucrose fractions. RNA was purified from nineteen 700µl sucrose fractions (15-45% sucrose) using the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument as described above. 2µl of each sample was electrophoresed using the High Sensitivity RNA ScreenTapes and Reagents (Agilent). RIN^e values are displayed for each eluate. Fractions 6 and greater have visible 28S and 18S rRNA bands, and the less intense band in the middle is likely the Luciferase Control RNA (~1,800 bases).

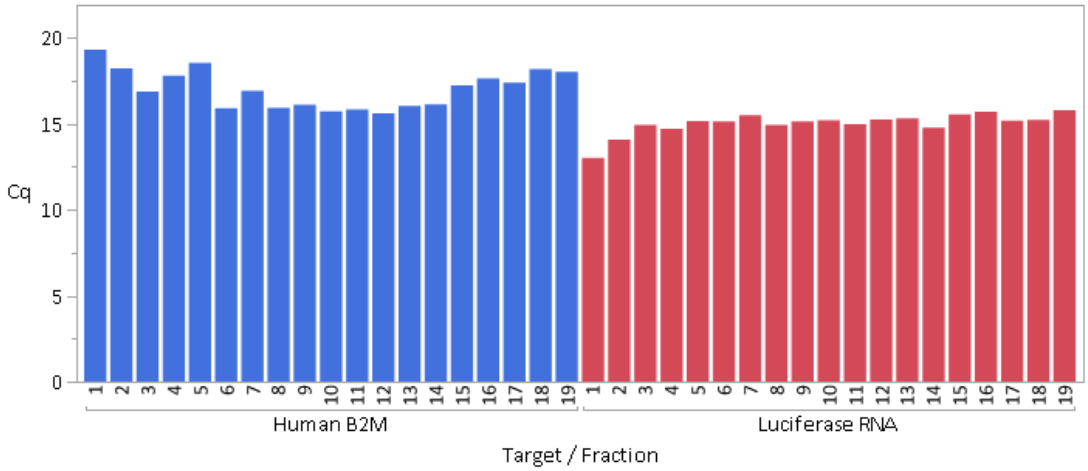

Figure 2. 1-Step RT-qPCR amplification of human-β2M RNA and Luciferase Control RNA extracted from 15-45% sucrose fractions. RNA was purified from nineteen 700µl sucrose fractions (15-45% sucrose) using the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument as described above. 2µl of each sample was amplified in duplicate with the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) using primers specific to human β2M RNA or the spiked Luciferase Control RNA. Bars represent average Cq value of duplicate amplifications.