Promega

Product Application

In collaboration with: neoteryx by TRAJAN

Automated RNA Purification from Harpera™ Microbiopsy™ Punches

Purify total RNA from skin microbiopsies using the Maxwell® RSC Instrument to obtain RNA suitable for amplification-based applications.

Kit: Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
Harpera™ Microbiopsy™ Punch (Trajan Scientific, Cat.# VH-01M20PI)

Analyses: RT-qPCR

Sample Type: Skin microbiopsies collected using the Harpera™ Microbiopsy™ Punch

Input: 1-3 punches

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

Contact Technical Services at: techserv@promega.com

Materials Required:

- Harpera™ Microbiopsy™ Punch (Trajan Scientific, Cat.# VH-01M20PI)
- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument or Maxwell® RSC 48 Instrument (Cat.# AS4500 or AS8500)

Protocol:

1. Collect 1-3 skin microbiopsies using the Harpera™ Microbiopsy™ Punch as described by the manufacturer. The manufacturer recommends maintaining the devices at room temperature for 30 minutes prior to disassembly for RNA purification.
2. Meanwhile, prepare solutions from the Maxwell® RSC miRNA Tissue Kit and chill on ice. Refer to the Technical Manual for more details.
    a. Prepare a working solution of 1-Thioglycerol/Homogenization Solution using 20µl of 1-Thioglycerol per 1ml of Homogenization Solution. 200µl of the prepared solution will be used per purification.
    b. Add 275µl of Nuclease-Free Water and 5µl of Blue Dye to a new vial of DNase I or thaw previously aliquotted DNase I solution on ice.
3. Dissassemble the device(s) as described in the Harpera™ User Guide and transfer the microbiopsy collector(s) to a clean 1.5ml tube on ice. If pooling microbiopsy specimens for RNA purification, place the required number of microbiopsy collectors in the same tube. Proceed immediately with RNA purification.
4. Add 200µl of cold 1-Thioglycerol/Homogenization Solution to each tube.
5. Vortex for 15-20 seconds to lyse cells.
6. Remove samples from ice and add 200µl of Lysis Buffer, 200µl of Lytic Enhancer, and 30µl of Proteinase K per sample and mix by vortexing. Note: Lysis Buffer, Lytic Enhancer, and Proteinase K can be prepared as a master mix immediately prior to use.
7. Incubate samples at room temperature for 10 minutes.
8. Meanwhile, prepare cartridges for the Maxwell® RSC miRNA Tissue Kit as described in the Technical Manual.
    a. Place cartridges to be used in the Maxwell® RSC deck tray, snap into place, and remove seals.
    b. Place a plunger in well 8 of each cartridge.

c. Place Elution Tubes in the front of the deck tray and add 60µl of Nuclease-Free Water to the bottom of each tube. (Reduced elution volumes are not compatible with this kit.)

d. Add 10µl of the prepared DNase I Solution to well 4.

9. When the incubation is complete, briefly centrifuge sample tubes and transfer the full lysate volume to well 1 of the cartridge. Pipet to mix.

    a. Optional: To maximize sample input, tubes may be briefly centrifuged a second time to collect residual lysate from the blades. Residual lysate can be added to well 1 of the appropriate cartridge.

10. Place the prepared deck tray in a Maxwell® RSC or Maxwell® RSC 48 Instrument and purify RNA using the miRNA Tissue method.

Results:

Amplifiable RNA was obtained from the majority of skin samples collected with a single Harpera™ Microbiopsy™ Punch and purified with the Maxwell® RSC miRNA Tissue Kit (Fig.1). RNA purified from 3 pooled Harpera™ Microbiopsy™ Punches resulted in consistent amplification of both targets for all samples (Fig.1).

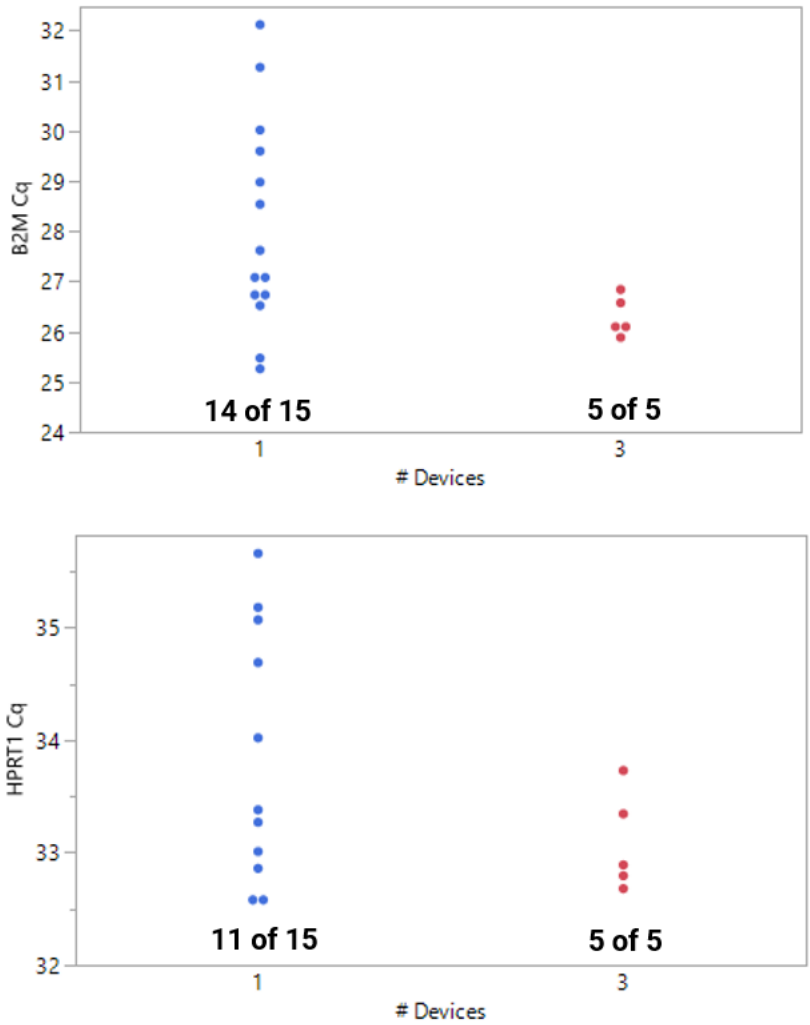

Figure 1. RNA amplification from individual or pooled Harpera™ Microbiopsy Punches and purified using the Maxwell® RSC miRNA Tissue Kit. Skin was sampled from the calf or forearm of five healthy individuals using Harpera™ Microbiopsy Punches. RNA was purified from either a single microbiopsy specimen (n=3 per individual) or from 3 pooled specimens (n=1 per individual) using the Maxwell® RSC miRNA Tissue Kit and eluted in 60µl of Nuclease-Free Water. **(Top)** 5µl of undiluted RNA was amplified using the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and primers specific to the high expression B2M gene. **(Bottom)** 5µl of undiluted RNA was amplified using the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and primers specific to the low expression HPRT1 gene. Cq values are shown for single amplification reactions per purification from a single specimen, or as a mean Cq for duplicate amplification reactions per purification from pooled specimens. The number of samples with amplification for each target is indicated in bold.

The Harpera™ Microbiopsy™ Punch is intended to enable the collection of a specimen from the cutaneous skin surface by a healthcare professional for clinical studies and is currently supplied globally as an investigational use only (IUO) product. The performance characteristics of this device have not been fully validated.