Maxwell® RSC simplyRNA Cells Kit and Maxwell® RSC simplyRNA Tissue Kit

Instructions for Use of Products AS1390 and AS1340.

Promega

Quick Protocol

Preparing Cell Samples for RNA Purification

Materials to be Supplied by the User

- microcentrifuge
- benchtop vortex mixer
- RNase-free, sterile, aerosol-resistant pipette tips

Solution Preparation

1-Thioglycerol/Homogenization Solution: To prepare a working solution, add 20µl of 1-Thioglycerol per milliliter of Homogenization Solution. 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement. Alternatively, add 600µl of 1-Thioglycerol to the 30ml bottle of Homogenization Solution. A volume of 200µl of 1-Thioglycerol/Homogenization Solution is needed for each sample. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

DNase I Solution: Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse DNase off the underside of the cap and swirl gently to mix; do not vortex. Add 5µl of Blue Dye to the reconstituted DNase I as a visual aid for pipetting. Dispense the DNase I Solution into single-use aliquots in nuclease-free tubes. Store reconstituted DNase I at –30°C to –10°C. Do not freeze-thaw reconstituted DNase I more than ten times.

Sample Preparation:

1. Trypsinize adherent cells following normal protocols.
2. Pellet cells at low speed (e.g., 300 × *g* for 3 minutes).
3. Remove medium.
4. Add 200µl of chilled 1-Thioglycerol/Homogenization Solution to the cell pellet and vortex until pellet is dispersed and cells appear lysed. A pipette may be used to disperse the pellets before vortexing. Store lysed cells on ice if there is a delay before processing.
5. Shortly before processing samples on the Maxwell® Instrument, add 200µl of Lysis Buffer (Part# MC501C) to 200µl of lysed cells. Vortex vigorously for 15 seconds to mix. Transfer all 400µl of lysate to well #1 (the largest well) of the Maxwell® RSC Cartridge.
6. Add 5µl of DNase I Solution to well #4 (yellow reagent). After adding the blue DNase I Solution, the reagent in well #4 will be green.

Preparing Tissue Samples for RNA Purification

Materials to be Supplied by the User

- small tissue homogenizer
- benchtop vortex mixer
- tube for homogenization
- RNase-free, sterile, aerosol-resistant pipette tips
- **optional:** heat block or water bath set to 70°C

Sample Preparation:

1. Homogenize the tissue sample in the chilled 1-Thioglycerol/Homogenization Solution until no visible tissue fragments remain. Homogenize an additional 15–30 seconds for complete homogenization. If foaming occurs, let sample settle on ice. The final volume of the homogenate added to the cartridge should be 200µl. Add 1-Thioglycerol/Homogenization Solution as needed to bring samples to a final volume of 200µl.

Sample Preparation (continued):

2. **Optional:** RNA yield from larger amounts of some tissues may be increased by heating homogenates at 70°C for 2 minutes, then allowing homogenates to cool (approximately 1 minute) before proceeding to Step 3. This is recommended for 10mg or more of liver tissue.

   Note: If the heat step is used, the purified RNA will migrate differently on native gels. Denaturing gels are recommended if the heating step is used.

3. Shortly before processing samples on the Maxwell® Instrument, add 200µl of Lysis Buffer (Part# MC501C) to 200µl of homogenate. Vortex vigorously for 15 seconds to mix. Transfer 400µl to well #1 (the largest well) of the Maxwell® RSC Cartridge.
4. Add 5µl of DNase I Solution to well #4 (yellow reagent). When using more than 5mg of tissues with high DNA content (e.g., liver or spleen), add 10µl of DNase I Solution to well #4. After adding the blue DNase I Solution, the reagent in well #4 will be green.

Maxwell® Automated RNA Purification

Cartridge Preparation

1. Place the cartridge to be used in the deck tray with well #1 (the largest well) facing away from the elution tube.
2. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing the cartridge in the instrument.
3. Place one plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.
4. Place 0.5ml Elution Tubes in the front of the Deck Tray. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube.

Notes:

1. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
2. Use only the Elution Tubes (0.5ml) provided with the kit; other tubes may be incompatible with supported Maxwell® Instruments.

Instrument Run on the Maxwell® Instruments

Follow the instrument run instructions in the *Maxwell® RSC simplyRNA Kits Technical Manual* #TM416.

Additional protocol information in Technical Manual #TM416, available online at: **www.promega.com**

