# Maxwell® 16 LEV simplyRNA Cells Kit and Maxwell® 16 LEV simplyRNA Tissue Kit

INSTRUCTIONS FOR USE OF PRODUCTS AS1270 AND AS1280.

## Preparation of Cell Samples for RNA Purification

### Materials to Be Supplied by the User

- centrifuge
- vortex mixer
- RNase-free, sterile, aerosol-resistant pipette tips

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 vortexting. Store lysed cells on ice if there is a delay before processing.
5. Shortly before processing samples on the Maxwell® 16 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 of the Maxwell® 16 LEV Cartridge (MCE). Well #1 is the closest to the cartridge label and farthest from the elution tube.
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.

## Preparation of Tissue Samples for RNA Purification

### Materials to Be Supplied by the User

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

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 additional homogenization solution as needed to bring samples to a final volume of 200µl.
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® 16 Instrument, add 200µl of Lysis Buffer (Part# MC501C) to 200µl of homogenate. Vortex vigorously for 15 seconds to mix. Transfer 400µl to well 1 of the Maxwell® 16 LEV Cartridge (MCE).
4. Add 5µl of DNase 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 to well #4. After the blue DNase I solution is added, the reagent in well #4 will be green.


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INSTRUCTIONS FOR USE OF PRODUCTS AS1270 AND AS1280.

## Solution Preparation, Cartridge Preparation and Instrument Setup

### Solution Preparation

**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:** 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 –20°C. Do not freeze-thaw reconstituted DNase I more than three times.

### Cartridge Preparation

Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack with the label side facing away from the Elution Tubes. 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 cartridges in the instrument.
**Note:** If you are processing fewer than 16 samples, center the cartridges on the platform.

1. Place an LEV Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
2. Place 0.5ml Elution Tubes in the front of the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. For a more concentrated eluate, as little as 30µl of nuclease-free water may be added to the elution tube, but the total amount of RNA recovered may be reduced.

   **Notes:**

   1. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
   2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® 16 Instrument.

### Instrument Run on the Maxwell® 16 Instrument (Cat.# AS2000 or AS3000)

1. Refer to the *Maxwell® 16 Instrument Operating Manual* #TM295 (AS2000) or #TM320 (AS3000) for detailed information. To run the simplyRNA protocol, the Maxwell® 16 firmware version ≥4.95 (AS2000) or ≥1.50 (AS3000) must be installed on the instrument and the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070) must be used. Using the original LEV magnetic rod will result in low yields.
2. Follow the instrument run instructions in the *Maxwell® 16 LEV simplyRNA Kits Technical Manual* #TM351. To run the simplyRNA protocol for AS2000 instruments, select “RNA”, select “simplyRNA”, then select “simplyRNA” once more on the Menu screen. To run the simplyRNA protocol for AS3000 instruments, select “RNA”, then select “simplyRNA” on the Menu screen.

Additional protocol information in Technical Manual #TM351, available online at:
**www.promega.com/protocols/**

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