Maxwell® RSC simplyRNA Tissue Kit

Cartridge and Sample Prep

- [ ] Identify samples to be processed.
- [ ] Clean work station & obtain and ice bucket
- [ ] Remove DNAase from -20°C and thaw on ice
- [ ] Remove samples from -80°C and thaw on ice.
- [ ] Change gloves before handling cartridges, Maxwell® CSC/ RSC Plungers and Elution Tubes to maintain an RNase-free environment during processing.
- [ ] Place the cartridges in the deck tray with the printed side facing away from the Elution Tubes. Press down to snap it into position. Note: Center cartridges on the deck.
- [ ] Carefully peel back the seal so that all plastic comes off the top of the cartridge.
- [ ] Place a Maxwell® CSC/RSC Plunger in the well closest to the Elution Tube (well #8).
- [ ] Place 0.5mL Elution Tubes in the front of the deck tray. Add 40µl of Nuclease-Free Water to the bottom of each Elution Tube.
- [ ] Add 5µl of blue DNase I Solution to well #4 (yellow reagent).
- [ ] Add 200µl of Lysis Buffer to the thawed punch sample. Vortex vigorously for 15 seconds to mix. Transfer all 400µl of lysate to the well farthest from the elution tube (well #8).

Running the Maxwell

- [ ] Turn on the Maxwell® RSC Instrument and Tablet PC.
- [ ] Touch the "Maxwell RSC button" to open the software.
- [ ] Touch the "Start" button to access the protocols.
- [ ] Select "Maxwell RSC SimplyRNA Tissue" and touch "Proceed".
- [ ] Enter the lot number and expiration date as prompted.
- [ ] If running < 16 samples, tap the blue bar to de-select a cartridge.
- [ ] Touch "Proceed" then "okay" and the door will open.
- [ ] Confirm that all checklist items have been performed.
- [ ] Transfer the deck tray with prepared cartridges onto the platform. It should "click" into place.
- [ ] Verify that samples were added to well #1, Elution Tubes have 30µl of Nuclease-Free Water and the caps are open, the Plungers are in well #8., and #4 is green.
- [ ] Touch the Start button to begin the extraction run. The platform will retract, and the door will close.

After the Maxwell Run Completes

- [ ] Follow on-screen instructions at the end of the method to open door. Verify that the plungers are located in well #8 of the cartridge at the end of the run.
- [ ] Remove the deck tray from the instrument. Remove Elution Tubes containing RNA, and close the tubes.
- [ ] Transfer 5µl RNA to a tube for analysis on the Bioanalyzer.

- [ ] Transfer 5µl RNA to a tube for analysis on the Quantas.
- [ ] Transfer remaining ~20µl RNA to a 1.5ml tube for labeled with the RNAseq ID for shipment to the GSAF.
- [ ] Remove the cartridges and plungers from the deck tray and discard, following your institutions recommended guidelines for disposal of hazardous material. Do not reuse reagent cartridges, plungers or Elution Tubes.

Other Useful Information

Where did these samples come from?

Tissue punches for this project were collected as described here. They were homogenized in 200µL 1-Thioglycerol/Homogenization Solution and stored at -80°C.

Solution Prep and Storage

1-Thioglycerol/Homogenization Solution

A volume of 200µl of 1-Thioglycerol/Homogenization Solution is needed for each sample. 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. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

Storage Note: Store the 1-Thioglycerol/Homogenization Solution at 2–10°C, where it is stable for up to 30 days. Upon receipt, remove 1-Thioglycerol and store at 2–10°C. Store the remaining kit components at room temperature (15–30°C). 1-Thioglycerol also can be stored at room temperature (15–30°C), where it is stable for up to 9 months.

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. DNase I solution maintains activity for up to 10 freeze-thaw cycles.