Maxwell® RSC RNA FFPE Kit

Instructions for Use of Product AS1440.

Quick Protocol

Preprocessing FFPE Section Samples

1. Place the FFPE tissue section into a 1.5ml microcentrifuge tube. If using slide-mounted tissue sections, scrape the section off the slide using a clean razor blade. Tap or centrifuge tube briefly to collect the sample at the bottom of the tube.

   Note: Use tissue sections (5–10 microns thick) ranging in size from 20mm^2 to 200mm^2 for a total of up to 2.0mm^3 of tissue.

2. Add 300µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
3. Heat the samples at 80°C for 2 minutes, then place samples at room temperature while the master mix is prepared.
4. Prepare a master mix of the Lysis Buffer, Proteinase K Solution and Blue Dye as shown below:

Reagents	Amount/Reaction	Reactions (n + 2)	Total
Lysis Buffer	224µl	n + 2	224 × (n + 2)µl
Proteinase K	25µl	n + 2	25 × (n + 2)µl
Blue Dye	1µl	n + 2	1 × (n + 2)µl

   For fewer than six samples, prepare enough master mix for n + 1 samples.

   Note: Use the master mix within 1 hour of preparation. Master mix cannot be stored for later use.

5. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.
6. Centrifuge sample tubes at 10,000 × *g* for 20 seconds to separate layers. If a pellet is present in the aqueous layer (lower blue layer), gently mix the aqueous phase with a pipette to resuspend the pellet.
7. Transfer the sample tubes to a 56°C heat block and incubate for 15 minutes.
8. Transfer the sample tubes to an 80°C heat block and incubate for 1 hour.
9. Remove the sample tubes from the heat block, and allow the samples to cool to room temperature for 15 minutes.
10. Prepare a DNase cocktail containing $MnCl_2$, DNase Buffer and reconstituted DNase I in the order shown below:

Reagents	Amount/Reaction	Reactions (n + 2)	Total
$MnCl_2$, 0.09M	26µl	n + 2	26 × (n + 2)µl
DNase Buffer	14µl	n + 2	14 × (n + 2)µl
DNase I	10µl	n + 2	10 × (n + 2)µl

    For fewer than six samples, prepare enough master mix for n + 1 samples.

11. Add 50µl of DNase cocktail to the blue, aqueous phase in each sample tube. Mix by pipetting 10 times.
12. Incubate sample tubes for 15 minutes at room temperature (15–30°C).
13. Centrifuge the sample tubes at full speed in a microcentrifuge for 2 minutes.
14. Immediately transfer the blue, aqueous phase to well #1 of a Maxwell® FFPE Cartridge.

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Quick Protocol

DNase I Preparation, Method Setup and Cartridge Preparation

DNase I Solution

Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I prior to use. Invert the vial to rinse DNase I off the underside of the cap and swirl gently to mix; do not vortex. Store reconstituted DNase I at –30°C to –10°C after use. DNase I solution maintains activity for up to 10 freeze-thaw cycles.

Maxwell® RSC Method Setup

Before using the Maxwell® RSC RNA FFPE Kit for the first time, the Maxwell® RSC FFPE RNA method must be installed on your instrument. The method is available at: **www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-rsc-fsc-software-firmware-methods/**

See the *Maxwell® RSC Methods Installation Technical Manual* #TM435 for instructions.

Cartridge Preparation

1. Place the cartridges to be used in the Maxwell® RSC/CSC Deck Tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes. Press down on the cartridge to snap it into position. Ensure both cartridge ends are fully seated in the deck tray. Carefully peel back the seal so that the entire seal is removed from the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed from the cartridges.
   Caution: Handle cartridges with care. Seal edges may be sharp.

   Note: If you are processing fewer than 16 samples, center the cartridges on the Deck Tray.

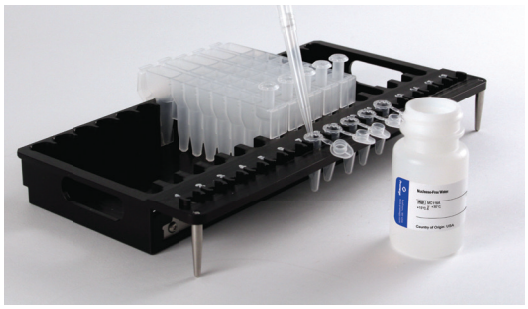

Figure 1. Setup and configuration of the Maxwell® RSC/CSC Deck Tray.

2. Place one plunger into well #8 of each cartridge.
3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the Maxwell® RSC/CSC Deck Tray.
4. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. The Elution Tubes must stay open during RNA purification.

   Note: Use only the CSC/RSC Plungers, Elution Tubes and Nuclease-Free-Water supplied with the Maxwell® RSC RNA FFPE Kit. Plungers for Maxwell® 16 LEV kits are not compatible with Maxwell® RSC Instruments. Other elution tubes may not be compatible with the Maxwell® RSC Instruments and may affect performance. Use of other elution buffers may affect RNA purification performance or downstream use.

Maxwell® RSC Instrument Run

1. Follow the instrument run instructions in the *Maxwell® RSC RNA FFPE Kit Technical Manual* #TM436.
2. Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 or the *Maxwell® RSC 48 Instrument Operating Manual* #TM510 for detailed information.

Additional protocol information in Technical Manual #TM436, available online at: **www.promega.com**

