

Maxwell® RSC RNA FFPE Kit

INSTRUCTIONS FOR USE OF PRODUCT AS1440.

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: 5–10 micron-thick tissue sections ranging in size from 20mm² to 200mm² for a total of up to 2.0mm³ of tissue can be used.
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:

Reagent	Amount/Reaction	Reactions (number to be run + 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 pipet 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 MnCl₂, DNase Buffer and reconstituted DNase I in the order shown below:

Reagent	Amount/Reaction	Reactions (number to be run + 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

11. Add 50µl DNase cocktail to the aqueous (blue) 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.

Note: For some difficult sample types (e.g., skin) incubating the sample in well #1 for 5 minutes before starting the Maxwell® method may increase RNA recovery.

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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/tools/maxwellrscmethod. 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.

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 the 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 the Maxwell® RSC Instrument. Other elution tubes may not be compatible with the Maxwell® RSC Instrument and may affect performance. Use of other elution buffers may impact RNA purification performance or downstream use.



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

Maxwell® RSC Instrument Run (Cat.# AS4500)

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 for detailed information.

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

Ordering and Technical Information

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