Maxwell® RSC Xcelerate DNA FFPE Kit

Instructions for Use of Product AS1510.

Promega

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: FFPE tissue sections up to a total input volume of 2.0mm^3 can be used. The Maxwell® RSC Xcelerate DNA FFPE Kit performance was evaluated by isolating DNA from FFPE tissue input volume of 0.02–2.0mm^3.

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 per Reaction	Reactions (number + 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. Do not store master mix 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. Remove the sample tubes from the heat block.
9. Add 25µl of Xcelerate Buffer (XB1) to the aqueous layer (lower, blue layer). Gently mix Xcelerate Buffer (XB1) into the lysate by pipetting. Mixing the lower aqueous layer (blue) and the Xcelerate Buffer (XB1; yellow) will result in a green color, indicating the Xcelerate Buffer (XB1) has been added and is sufficiently mixed.

   Note: Add exactly 25µl of the Xcelerate Buffer (XB1). Adding more or less of the Xcelerate Buffer (XB1) may negatively affect DNA yields.

10. Transfer the sample tubes to an 80°C heating block and incubate for 30 minutes.

    Note: Incubate the samples for exactly 30 minutes. Incubating for longer or shorter times may negatively affect DNA yields.

11. Remove the sample tubes from the heat block and cool the samples to room temperature for 5 minutes.
12. Add 10µl of RNase A Solution to the aqueous (green) phase in each sample tube. Mix by pipetting.
13. Incubate for 5 minutes at room temperature (15–30°C). During the incubation, prepare cartridges (see the next page for instructions).
14. Centrifuge the sample tubes at full speed in a microcentrifuge for 5 minutes.
15. Immediately transfer the green aqueous phase containing the DNA to well #1 of a Maxwell® FFPE Cartridge.

    Note: Take care to avoid the pellet and any insoluble tissue debris. Transfer of insoluble tissue into well #1 of the cartridge may negatively affect DNA yields.

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Method Setup and Cartridge Preparation

Maxwell® RSC Method Setup

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

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

Cartridge Preparation

1. Place the cartridges to be used in the 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.
2. Place one plunger into well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray.
4. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube.
   Note: Use only the CSC/RSC Plungers, Elution Tubes and Nuclease-Free-Water supplied with the Maxwell® RSC Xcelerate DNA FFPE Kit. Plungers for Maxwell® 16 LEV kits are not compatible with Maxwell® RSC Instruments. Other elution tubes may not be compatible with Maxwell® RSC Instruments and may affect performance. Use of other elution buffers may impact DNA purification performance or downstream use.
5. Proceed to the next section, Instrument Run on Maxwell® RSC Instruments.

Figure 1. Setup and configuration of deck trays. Nuclease-Free Water is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

Instrument Run on Maxwell® RSC Instruments (Cat.# AS4500, AS8500)

1. Follow the instrument run instructions in the *Maxwell® RSC Xcelerate DNA FFPE Kit Technical Manual* #TM744.
2. Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 or *Maxwell® RSC 48 Instrument Operating Manual* #TM510 for detailed information.