Maxwell® RSC XtractAll FFPE DNA/RNA Kit

Instructions for Use of Product AS1570.

Quick Protocol

Preparing FFPE Tissue Samples for Sequential DNA and RNA Extraction

To obtain optimal kit performance, use up to 20µm total thickness of FFPE tissue sections. Multiple sections can be combined in one sample tube for extraction with the maximum thickness of combined sections ≤80µm. Sections thicker than 20µm will affect the Proteinase K digestion and result in low yields. You should optimize the number of sections and section thickness for compatibility with laboratory downstream analysis.

Materials to Be Supplied by the User

- microcentrifuge
- benchtop vortex mixer
- pipettors and pipette tips for sample preprocessing and transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., Microtubes, 1.5ml; Cat.# V1231)
- heat blocks set at 56°C and at 90°C
- FFPE tissue samples (**Note:** Samples should be stored at room temperature [15–30°C].)
- isopropanol, ≥99.5% Molecular Biology Grade
- razor blades (**Note:** Use caution when scraping samples from the slide with a razor blade.)

Note: As needed, reconstitute a lyophilized vial of DNase I with 275µl of Nuclease-Free Water and 15µl of Blue Dye. Invert the vial to recover any DNase I from the underside of the cap and swirl gently to mix; **do not** vortex. Store reconstituted DNase I at –30°C to –10°C. DNase I solution maintains activity for up to 10 freeze-thaw cycles.

1. Place the FFPE tissue section(s) into a 1.5ml microcentrifuge tube. If you are using slide-mounted FFPE tissue sections, scrape the section(s) off the slide using a clean razor blade.
2. Add 500µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
3. Heat the samples at 90°C for 5 minutes. Place the samples at room temperature while the master mix is prepared as described in Step 4.
4. Immediately before use, prepare a master mix of the Lysis Buffer, Proteinase K and Blue Dye as shown below:

Reagent	Amount per Reaction	Reactions (Number to Be Run + 2)	Total
Lysis Buffer	224µl	n + 2	224µl × (n + 2)
Proteinase K	25µl	n + 2	25µl × (n + 2)
Blue Dye	1µl	n + 2	1µl × (n + 2)

5. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.

   Note: Do not store any remaining unused master mix.
6. Centrifuge sample tubes at 10,000 × *g* for 20 seconds to separate the layers. If a pellet is present in the aqueous layer (lower, blue layer), gently mix with a pipette tip to disperse the pellet. Avoid disturbing the mineral oil and aqueous layers in the tube as much as possible.
7. Transfer the sample tubes to a 56°C heat block and incubate for 15 minutes.
8. Transfer the sample tubes to a 90°C heat block and incubate for 1 hour. During this incubation, prepare cartridges as described below.

First Run: Maxwell® Automated DNA Purification

Cartridge Preparation

1. Place the cartridge to be used in the deck tray with well #1 (the largest well in the cartridge) facing away from the elution position, which is the numbered side of the tray.
2. 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 the cartridge in the instrument.
3. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.
4. Place an empty elution tube into the elution tube position for each cartridge. Add 30–100µl of Nuclease-Free Water to the bottom of each elution tube.

   Note: Use only the Elution Tubes (0.5ml) provided in the kit; other tubes may be incompatible with supported Maxwell® Instruments.

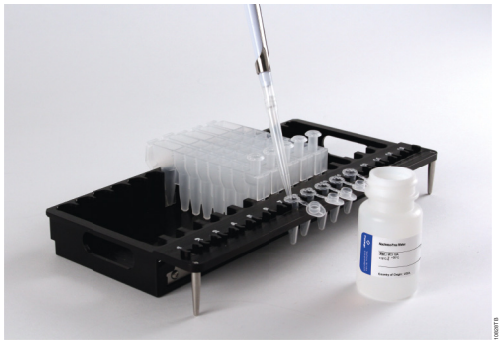

Figure 1. Setup and configuration of deck trays. Nuclease-Free Water is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

5. After the end of the 1-hour incubation, transfer the blue aqueous phase to well #1 of the Maxwell® RSC Cartridge (RSCR).

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the blue aqueous phase to the cartridge and extract within 30 minutes after completing incubation.

Instrument Run on the Maxwell® Instruments

Follow the instrument setup and run instructions in the *Maxwell® RSC XtractAll FFPE DNA/RNA Kit Technical Manual* #TM762.

Between Run Instructions

Between the first and second extraction runs for the sequential method, you need to perform the following steps:

1. Follow on-screen instructions at the end of the method to open the door. Verify that the plungers are located in well #8 of the cartridges at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the technical manual appropriate to your Maxwell® Instrument to perform a Clean Up process to unload the plungers. To prepare for the Sequential RNA extraction run, a new cartridge setup screen will show.
2. Cap and remove the Elution Tubes containing DNA immediately following the run to prevent eluate evaporation.
3. At the end of the Sequential DNA extraction run, the resin is deposited in well #2 to prepare for the Sequential RNA extraction run.

   Notes:

   a. Do not remove or dispose of cartridges or plungers from the deck tray. They will be reused for the Sequential RNA extraction.

   b. Proceed to Sequential RNA extraction within 2 hours after completing the Sequential DNA extraction.

4. A cartridge setup screen will be shown, indicating the sample positions and identifiers entered before the first DNA extraction run. If necessary, this information can be edited to reflect any changes to the cartridges being processed by touching the **Enable Editing** button.
5. Touch the **Proceed** button to bring up the 'Extraction Checklist' screen.

Second Run: Maxwell® Automated RNA Purification

Cartridge Preparation

1. Place an empty elution tube into the elution tube position for each cartridge. Add 30–100µl of Nuclease-Free Water to the bottom of each elution tube.

   Note: Use only the Elution Tubes (0.5ml) provided in the kit; other tubes may be incompatible with supported Maxwell® Instruments.
2. Immediately before use, prepare a cocktail of $MnCl_2$ and DNase I as shown below:

Reagent	Amount per Reaction	Reactions (Number to Be Run + 2)	Total
$MnCl_2$, 0.09M	17µl	n + 2	17µl × (n + 2)
DNase I (with Blue Dye)[1]	10µl	n + 2	10µl × (n + 2)

[1]Store remaining reconstituted DNase I with Blue Dye at –30°C to –10°C.

3. Add 27µl of DNase cocktail to well #7 of each cartridge.

   Note: Do not store excess unused DNase cocktail.
4. Add 500µl of 100% isopropanol to well #1.

Instrument Run on the Maxwell® Instruments

Follow the instrument setup and run instructions in the *Maxwell® RSC XtractAll FFPE DNA/RNA Kit Technical Manual* #TM762.

Additional protocol information is in Technical Manual #TM762, available online at: **www.promega.com**