Maxwell® RSC XtractAll FFPE DNA/RNA Kit

Instructions for Use of Product AS1570.

Quick Protocol

Preparing FFPE Tissue Samples for Total Nucleic Acid 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.)

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.

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Maxwell® Automated Total Nucleic Acid 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.
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.
6. Add 500µl of 100% isopropanol to well #1.

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 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**