# Maxwell® 16 LEV RNA FFPE Kit

INSTRUCTIONS FOR USE OF PRODUCT AS1260.

## Preparation of FFPE Samples for RNA Purification

### Sample Information

The Maxwell® 16 LEV RNA FFPE kit is only intended for use with mammalian FFPE tissue samples fixed with 10% neutral-buffered formalin. The tissue input was evaluated by isolating RNA from FFPE mammalian (mouse and human) tissue samples ranging in thickness from 5–10 microns with a size range of 20mm$^2$ to 200mm$^2$ for a total of up to 2.0mm$^3$.

### Materials to Be Supplied by the User

- microcentrifuge
- pipettors and pipet tips
- 1.5–2.0ml tubes and a 5–15ml tube for the master mix
- heat blocks (56°C and 80°C)
- FFPE tissue sections
- razor blades (for sections on slides)

### DNase I Preparation

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 –10°C to –30°C after use. DNase I solution maintains activity for up to 10 freeze-thaw cycles.

### Preprocessing Samples

1. Place tissue section into 1.5ml microcentrifuge tube. If using slide-mounted tissue sections, scrape section off of slide using a clean razor blade.
2. Add 300µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
3. Heat the samples at 80°C for 2 minutes. Place the samples at room temperature while the master mix is prepared.
4. Prepare a master mix of the Lysis Buffer, Proteinase K 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. 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 aqueous phase with pipet to resuspend the pellet.
7. Transfer the sample tubes to 56°C heat block and incubate for 15 minutes.
8. Transfer the sample tubes to 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.

(protocol continued on next page)



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10. Prepare a DNase cocktail containing $MnCl_2$, 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 |
| For fewer than six samples, prepare enough master mix for n + 1 samples. | | | |

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 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.

## Cartridge Preparation

1. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack with the label side facing away from the Elution Tube.
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 cartridges in the instrument.
   **Note:** If you are processing fewer than 16 samples, center the cartridges on the cartridge rack.
3. Place an LEV Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
   **Note:** Use only the plungers provided in the Maxwell® 16 LEV RNA FFPE Kit. Plungers for the Maxwell® CSC kits are not compatible with the Maxwell® 16 Instrument.
4. Place 0.5ml Elution Tubes in the front of the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube.
   **Notes**
   a. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
   b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® 16 Instrument.

## Instrument Run on the Maxwell® 16 Instrument (Cat.# AS2000 or AS3000)

1. Refer to the *Maxwell® 16 Instrument Operating Manual* #TM295 (AS2000) or #TM320 (AS3000) for detailed information. To run the RNA FFPE protocol the Maxwell® 16 firmware version ≥4.95 (AS2000) or ≥1.50 (AS3000) must be installed on the instrument, and the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070) must be used.
2. Follow the instrument run instructions in the *Maxwell®16 LEV RNA FFPE Kit Technical Manual* #TM408.

**Ordering and Technical Information**
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