

Isolation of: **Total RNA**

Sample Type: formalin-fixed paraffin-embedded (FFPE) tissue

Kit: RSC RNA FFPE Kit **AS1440**

Sample preparation:

- 1.) Add **275 µl** 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 -20°C after use (maximum of 10 freeze-thaw cycles).
- 2.) Transfer sample material (FFPE sections with thickness of 5-10 µm with a total tissue volume of up to 2,0 mm³) into a 1,5 ml microcentrifuge tube and add **300 µl** of mineral oil to the sample tubes.
- 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 **224 µl** Lysis Buffer, **25 µl** Proteinase K and **1 µl** Blue Dye (n+2 preparations for n reactions; for n = 1 to 5 prepare n+1).

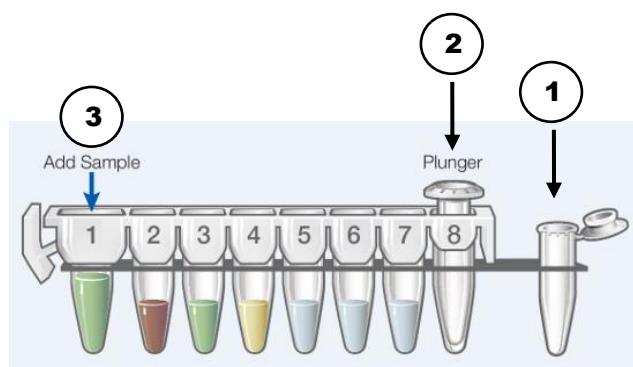
NOTE: Use the master mix within 1 hour of preparation!

- 5.) Add **250µl** of master mix to each sample tube and vortex for 5 seconds.
- 6.) Centrifuge sample tubes at 10.000 x g for 20 seconds to separate layers. If a pellet is present in the aqueous layer (lower, blue layer), gently mix aqueous phase by pipetting 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.
- 10.) Prepare a DNase cocktail in the following order: **26 µl** MnCl₂, **14 µl** DNase Buffer and **10 µl** reconstituted DNase I (n+2 or n+1 preparations, similar to master mix).
- 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 of a Maxwell® FFPE cartridge (see next page).

Please note: This protocol is an instruction advice/ recommendation but cannot replace in-house evaluation. Further information can be found in the technical manual available online at www.promega.com/resources/protocols

Extraction:

- 1.) Place the cartridge into the sample rack and remove the seal.
- 2.) Place the supplied elution tubes into the designated position of the sample rack and add **50µl** of Nuclease-Free Water to the elution tube (s. **1**).
- 3.) Place the plunger in the indicated position of the cartridge (s. **2**).
- 4.) Transfer the whole aqueous phase into well 1 of the Maxwell cartridge (s. **3**). Do not transfer eventually remaining paraffin of the sample tube into the Maxwell-rack!
- 5.) Select the appropriate program for the **RNA FFPE Kit** and start the run.
- 6.) After extraction your sample is ready-to-use for your downstream applications.



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