Isolation of: RNA

Sample Type: Formalin-fixed paraffin-embedded tissue

Kit: Maxwell® 16 FFPE Plus LEV RNA Purification Kit **AS1260**

NOTE: firmware version 4.95 or higher for AS2000 and 1.50 or higher for AS3000 required

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 –10°C to –30°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 mm^3) 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** $MnCl_2$, **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 (3) of a Maxwell® FFPE cartridge (see backpage).

Revised: March 2014

Extraction:

1.) Place the LEV-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 tubes (see (1)).

3.) Place the plunger in the indicated position of the cartridge (see (2)).

4.) Transfer the whole aqueous phase into well 1 of the Maxwell cartridge (see image below (3)). Do not transfer eventually remaining paraffin of the sample tube into the Maxwell-rack!

5.) Select **LEV** configuration of the Maxwell and select method: **RNA → FFPE**. Start run.

6.) After extraction your sample is ready-to-use for your downstream applications.