

**Isolation of:****Genomic DNA****for detection of:****Human target genes****Sample Type:**

Formalin-fixed paraffin-embedded (FFPE) tissue

**Kit:**

RSC DNA FFPE Kit AS1450

**Sample preparation:**

- 1.) Transfer your material (5 - 10  $\mu\text{m}$  thick FFPE sections, max. 2  $\text{mm}^3$ ) into a suitable microtube and overlay it with **450  $\mu\text{l}$**  of mineral oil and vortex for 10 s.
- 2.) Incubate the sample 2 min at 80°C and place the sample at room temperature.
- 3.) Meanwhile, prepare the following Master Mix:

|                              | <i>Per Sample</i>                   | <i>n Samples</i>                            |
|------------------------------|-------------------------------------|---|
| <i>Lysis Buffer</i>          | <b>224 <math>\mu\text{l}</math></b> | <b>224 x (n+2) <math>\mu\text{l}</math></b> |
| <i>Proteinase K-Solution</i> | <b>25 <math>\mu\text{l}</math></b>  | <b>25 x (n+2) <math>\mu\text{l}</math></b>  |
| <i>Blue Dye</i>              | <b>1 <math>\mu\text{l}</math></b>   | <b>1 x (n+2) <math>\mu\text{l}</math></b>   |

*Note: For n = 1 – 5 n+1 preparations are sufficient.*

*Use the Master Mix within 1 hour of preparation, do not store.*

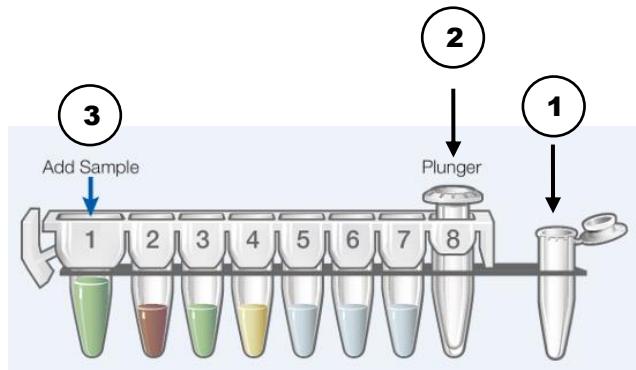
- 4.) Add **250  $\mu\text{l}$**  Master Mix to the sample and vortex some seconds
- 5.) Centrifuge the sample 20 s at 10.000  $\times$  g to separate layers. If a pellet is present in the aqueous layer (lower, blue layer), gently mix aqueous phase by pipetting to resuspend the pellet.
- 6.) Incubate the sample overnight at 56°C and subsequently for 30 min at 80°C.  
*Alternative: Depending on the type and age of the FFPE samples an incubation at 70°C overnight might be beneficial.*
- 7.) Remove the sample tubes from the heat block, and allow the samples to cool to room temperature for 5 minutes.
- 8.) Pipet 10  $\mu\text{l}$  RNase A-Solution into the aqueous phase and mix by pipetting.
- 9.) Incubate the samples for 5 min at room temperature. Meanwhile, prepare the cartridges (see Extraction 1.-3.)
- 10.) Centrifuge 5 min at full speed in a microcentrifuge.

**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](http://www.promega.com/resources/protocols)

**Extraction:**

- 1.) Place the cartridge to be used into the Maxwell cartridge rack and remove the seal.
- 2.) Place one of the supplied elution tubes into the sample rack and add **50 – 100 µl** of the supplied elution buffer (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 **3**).  
Do not transfer eventually remaining paraffin of the sample tube into the Maxwell rack!
- 5.) Select the appropriate program for the **DNA FFPE Kit** and start the run.
- 6.) After the extraction your sample is ready-to-use for your downstream applications.



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