

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 µm thick FFPE sections, max. 2 mm³) into a suitable microtube and overlay it with **450 µl** 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>	224 µl	224 x (n+2) µl
<i>Proteinase K-Solution</i>	25 µl	25 x (n+2) µl
<i>Blue Dye</i>	1 µl	1 x (n+2) µl

Note: For $n = 1 - 5$ $n+1$ preparations are sufficient.

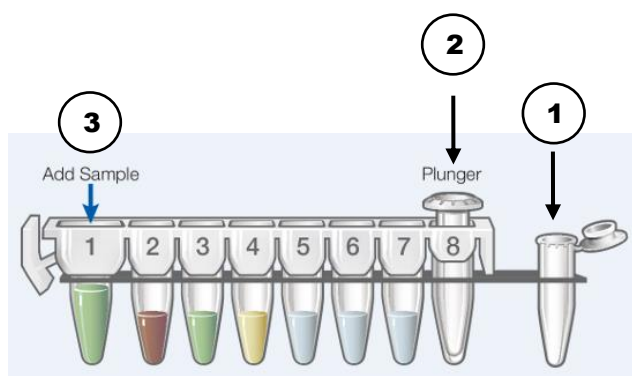
Use the Master Mix within 1 hour of preparation, do not store.

- 4.) Add **250 µl** Master Mix to the sample and vortex some seconds
- 5.) Centrifuge the sample 20 s at 10.000 x 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 µl 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

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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