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SONOPULS Application B-210

Disruption of FFPE tissue for DNA isolation

Introduction

The disruption of FFPE tissue is an essential step for DNA isolation and the conventional method is very time-consuming. The description is considered to be universally valid and is not linked to the isolation kits used.

Task

5 µm-thin FFPE tissue sections need to be disrupted as quickly and efficiently as possible for subsequent DNA isolation. Ultrasonic treatment can increase the DNA yield and cut the processing time.

Procedure

The tissue sections are deparaffinised and thawed for 1 hour at 56 °C with a lysis buffer and proteinase K mixture in a 1.5-ml tube, before being sonicated in the same container.

Method parameters

Device	HD 4200	HD 3200	HD 2200 HD 2200.2
Probe	BR 30		
Sonication vessel (dimensions + design)	1.5 ml tube, dist. water		
Amplitude [%]	50	48	Max. 52
Immersion depth Probe [cm]	Sample must not foam.		
Pulsation ON/OFF [s]	None		
Sonication time [min]	10 min ± 5 min each according to degree of disruption (visual control)		
Cooling (yes/no – sample temperature)	Brief cooling at room temperature after 10 min		

Cleaning

The sample can be further processed as usual. Remove the water from the beaker resonator.

Notes

The sonication may result in an (even) stronger fragmentation of the DNA to be isolated, which may make them unusable for PCR analyses with larger amplified products. For this reason, sonication should only be continued as long as needed and the thus-obtained DNA used to achieve the shortest-possible (≤300 bp) amplified products. In the case of especially important samples, a conventional batch without sonication should also be prepared as a precaution.

Example tables

Type of sample	DNA concentration without ultrasound	Analysis results following ultrasonic treatment CFU/g yeast
Breast tissue **Sample 1** **Sample 2**	 25.8 ng/µl 19.9 ng/µl	 31.5 ng/µl 28.3 ng/µl
Lung tissue **Sample 1** **Sample 2**	 40.6 ng/µl 39.3 ng/µl	 42.8 ng/µl 43.4 ng/µl
Colon tissue **Sample 1** **Sample 2**	 46.4 ng/µl 48.8 ng/µl	 48.8 ng/µl 49.6 ng/µl

Conclusion – Advantages

The long digestion times (often overnight) of FFPE tissues in a mix consisting of lysis buffer and proteinase K can be strongly accelerated with ultrasound treatment, allowing for fast, subsequent DNA isolation and further processing with PCR, especially in the case of urgent samples.

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