Product Application

DNA Purification from FFPE tissue samples on the KingFisher™ Flex

To isolate DNA from formaldehyde-fixed paraffin-embedded tissues on the KingFisher™ Flex in a 96-well format.

Kit: Maxwell® HT FFPE DNA Kit Custom (Cat. #AX4350)

Analyses: QuantiFluor® dsDNA Dye, GoTaq® qPCR Master Mix

Sample Type(s): FFPE mouse tissue curls

Input: 10µm sections

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, please contact **techserv@promega.com.**

Materials Required:

- KingFisher™ Flex Purification System
- KingFisher™ Deep Well 96 Plates (Thermo Scientific Cat.# 95040460)
- KingFisher™ 96 Tip Comb for Deep Well Magnets (Thermo Scientific Cat.# 97002534)
- 80% Ethanol
- Heat Block

Protocol:

Plate Set Up:

Plate #	Type	Components/Volumes
Plate 1	Binding	250µl lysate, 720µl Binding solution (MC114), 30µl Magnetic Particles (A200)
Plate 2	Wash 1	500µl Wash Solution 1 (MC116)
Plate 3	Wash 2	300µl Wash Solution 2 (MC117)
Plate 4	Wash 3	300µl Wash Solution 2 (MC117)
Plate 5	Wash 4	300µl 80% Ethanol (Make fresh)
Plate 6	Elution	100µl Nuclease-Free Water
Plate 7	Tip	Tip Comb

Pre-Processing:

1. Add 300µl of mineral oil to 5-10µm section in a 1.5ml microcentrifuge tube.
2. Heat samples for 80°C for 2 minutes. Place samples at room temperature while the lysis master mix is prepared.
3. Prepare lysis master mix for n+2 samples as indicated below.
    a. Lysis Buffer 224µl per sample
    b. Proteinase K 25µl per sample
    c. Blue Dye 1µl per sample
4. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.
5. Centrifuge at 10,000 *x g* for 20 seconds to separate layers.
6. Heat samples to 56°C on a heat block for 30 minutes.
7. Heat samples to 80°C on a heat block for 4 hours.

8. Transfer samples to the bench and allow the sample to cool to room temperature for 5 minutes.
9. Add 10µl of RNase A to the aqueous blue phase in each sample tube. Mix sample by pipetting.
10. Incubate for 5 minutes at room temperature.
11. Centrifuge samples at max speed in a microcentrifuge for 5 minutes.
12. Immediately transfer the blue, aqueous phase containing DNA to the well of plate 1 (~250µl).
13. Run the FFPE_DNA_96_v1_2 Protocol on the KingFisher™ Flex Purification System. Please contact Technical Services for method and more details.

Example Data:
10µm FFPE sections of mouse liver or brain were processed with the protocol above until step 11. Lysates from 7 sections from each tissue were pooled and 250µl of pooled lysate was added to the well of a KingFisher™ 96 Deep Well Plate for each tissue in triplicate. Samples were then run on the KingFisher™ Flex Purification System.

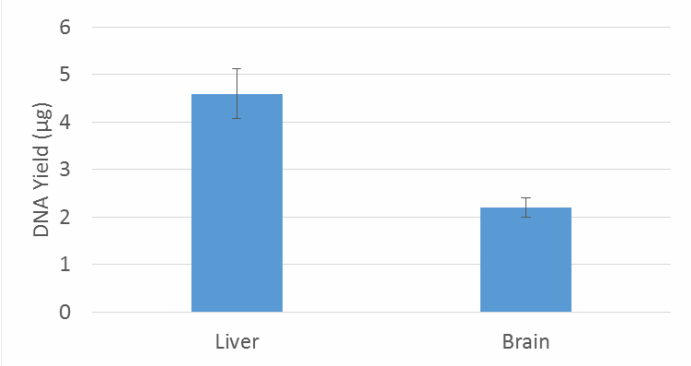

Figure 1. Purified DNA yield from FFPE tissue curls. DNA concentration was measured by fluorescent dye with QuantiFluor® ONE dsDNA (Cat.# E4871) on a Quantus™ Fluorometer (Cat.# E6150). Shown are the average ± standard deviation of n=3 for each condition.

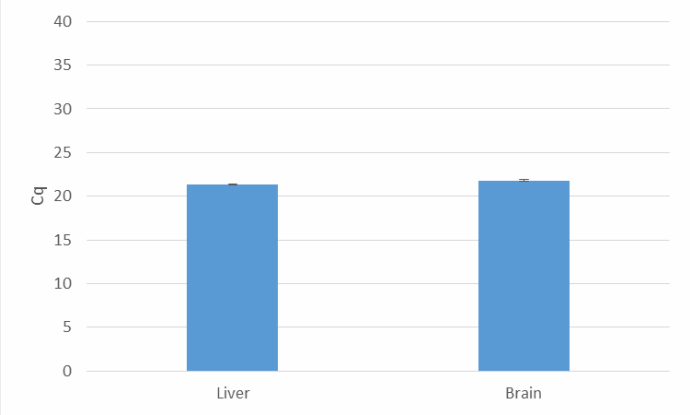

Figure 2. Cq values from amplification of DNA isolated from FFPE tissue curls. DNA was amplified with mouse DNA specific primers targeting Beta Actin. Shown are the average ± standard deviation of n=3.