Promega

Product Application

DNA purification from Hematoxylin and Eosin Stained FFPE Tissue using the Maxwell® RSC Instrument

Isolate high quality, amplifiable DNA from Hematoxylin and Eosin (H&E) stained FFPE tissue sections using the Maxwell® RSC DNA FFPE Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)

Analyses: Dye-based quantitation, qPCR

Sample Type(s): H&E stained FFPE tissue sections

Input: 1 5-10 µm FFPE tissue section

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)
- Harris Hematoxylin (Fisher Scientific, Cat.# SH26-500D)
- Eosin Y (Fisher Scientific, Cat.# 2845-16)
- Xylenes
- 100% ethanol
- Ultrapure water

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, see Technical Manual TM437, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

H&E Staining:

1. Warm slides at 65°C for 5 minutes to soften paraffin.
2. Incubate slides in 3 washes for 5 minutes in xylenes.
3. Incubate slides in 3 washes for 2 minutes in 100% ethanol.
4. Rinse slides in ultrapure water for 5 minutes.
5. Incubate slides in hematoxylin for 1 minute.
6. Rinse slides for 1 minute in ultrapure water.
7. Incubate slides in Eosin Y for 30 seconds.
8. Incubate slides in 3 washes for 2 minutes in 100% ethanol.
9. Incubate slides in 3 washes for 5 minutes of xylenes.
10. Mount and image or scrape tissues into 1.5ml tube for DNA isolation.

DNA Purification:

1. Add 300µl of mineral oil to scraped samples in 1.5ml tube. Incubate 2 minutes at 80°C.
2. Add 250µl of a master mix containing 224µl Lysis Buffer, 25µl Proteinase K Solution, and 1µl Blue Dye to each sample. Vortex samples for 5 seconds.
3. Spin for 2 minutes at 10,000 x *g* for 20 seconds.
4. Incubate for 30 minutes at 56°C.
5. Incubate for 4 hours at 80°C.
6. Allow samples to return to room temperature for 5 minutes. Add 10µl RNase A Solution to each sample and mix by pipetting.
7. Incubate for 5 minutes. Centrifuge at maximum speed for 5 minutes.
8. Transfer blue aqueous layer to well #1 of the Maxwell® FFPE cartridge. Run on the FFPE DNA protocol on the Maxwell® RSC Instrument.

Results:

Table 1. Concentration of DNA purified from Hematoxylin and Eosin Stained FFPE Tissue using the Maxwell® RSC DNA FFPE Kit (Cat.# AS1450) on the Maxwell® RSC Instrument (Cat.# AS4500). Concentration of DNA purified from human heart FFPE tissue sections (Biochain, Cat. #T2234122), assessed by the QuantiFluor® ONE dsDNA System (Cat.# E4871) on the Quantus™ Fluorometer (Cat.# E6150) and by qPCR using the GoTaq® qPCR Master Mix (Cat.# A6001), GAPDH primers and Human Genomic DNA (Cat.# G3041). Concentrations are shown in nanograms/µl for each replicate.

	DNA Concentration (ng/µl)	
	QuantiFluor®	qPCR
un-stained FFPE	0.43	0.41
	1.23	0.78
	0.65	0.21
H&E stained FFPE	0.95	0.43
	0.86	0.48
	0.98	0.43

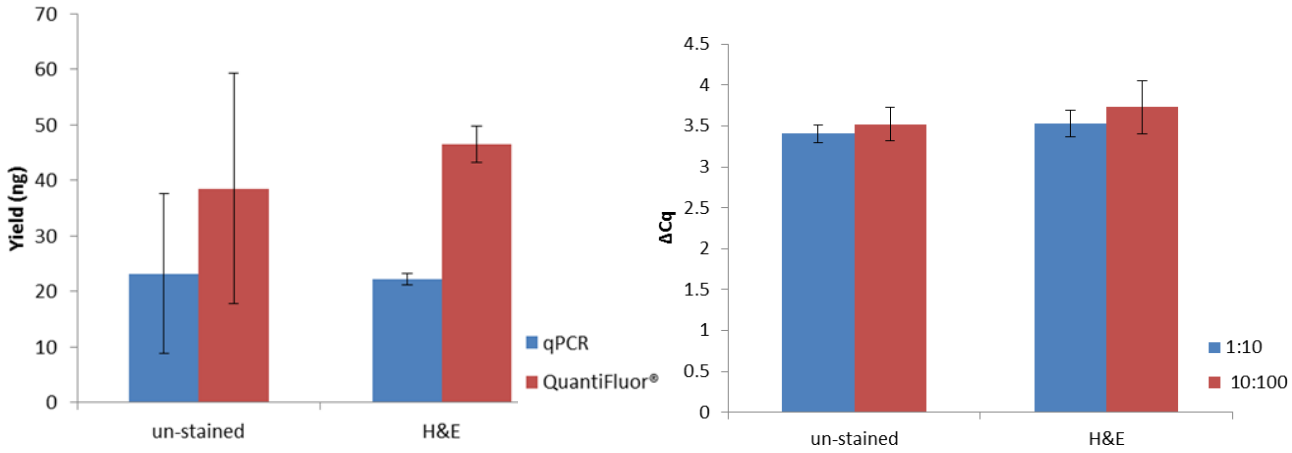

Figure 1. Yield of DNA purified from Hematoxylin and Eosin Stained FFPE Tissue using the Maxwell® RSC DNA FFPE Kit (Cat.# AS1450) on the Maxwell® RSC Instrument (Cat.# AS4500). Left: DNA yield from human heart FFPE tissue sections, assessed by the QuantiFluor® ONE dsDNA System (Cat.# E4871) on the Quantus™ Fluorometer (Cat.# E6150) and by qPCR using the GoTaq® qPCR Master Mix (Cat.# A6001), GAPDH primers and Human Genomic DNA (Cat.# G3041). **Right:** ΔCq values. Changes in Cq values of serially diluted samples indicate no inhibition. Note: A target amplified at 100% efficiency will give a ΔCq=3.3 between ten-fold dilutions.