

Testing FFPE Lysate Storage for TNA Extraction with Maxwell® FFPE RNA Kit

Tested DNA and RNA yield with storage of FFPE lysate after decrosslinking and prior to processing the samples with the Maxwell® RSC RNA FFPE Kit

Kit:	Maxwell® RSC RNA FFPE Kit (Cat.# AS1440)
Analyses:	qPCR, RT-qPCR
Sample Type(s):	SureShot™ ALK RET ROS1 Fusion Control Cells
Input:	Single Curls
Materials Required:	<ul style="list-style-type: none">▪ Maxwell® RSC RNA FFPE Kit (Cat.# AS1440)▪ Maxwell® RSC Instrument (Cat.# AS4500)▪ Thermomixer or heat blocks set to 56°C and 80°C▪ 1.5ml tubes▪ Microcentrifuge

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 TM436, available at:
www.promega.com/protocols or
contact Technical Services at:
techserv@promega.com

Protocol:

1. Samples were transferred to 1.5ml Eppendorf tubes from the original skirted sample tubes (for better heat transfer).
2. Processed samples through step #9 of the technical manual (TM436)
3. Stored samples for conditions B (at RT), C (in well #1 of the Maxwell® RSC RNA FFPE Cartridge at 4°C), and D (at -20°C) for 4h, as described above.
4. Omitted the DNase treatment for all the samples, for a total nucleic acid (TNA) protocol.
5. Added the aqueous phase to well #1 and processed samples for condition A right away, using FFPE RNA method on the Maxwell® RSC Instrument, with a 50µl elution volume.
6. After the indicated incubation/storage time, the samples were all brought to room temperature (~30 minutes) and then aqueous phase was added to well #1 of the Maxwell® RSC cartridge and samples were processed using the FFPE RNA method on the Maxwell® RSC Instrument with a 50µl elution volume.
7. Assessed the DNA and RNA yields by qPCR with 500nM GAPDH (DNA specific) primers and RT-qPCR with 500nM B2M (RNA specific) primers.

Results:

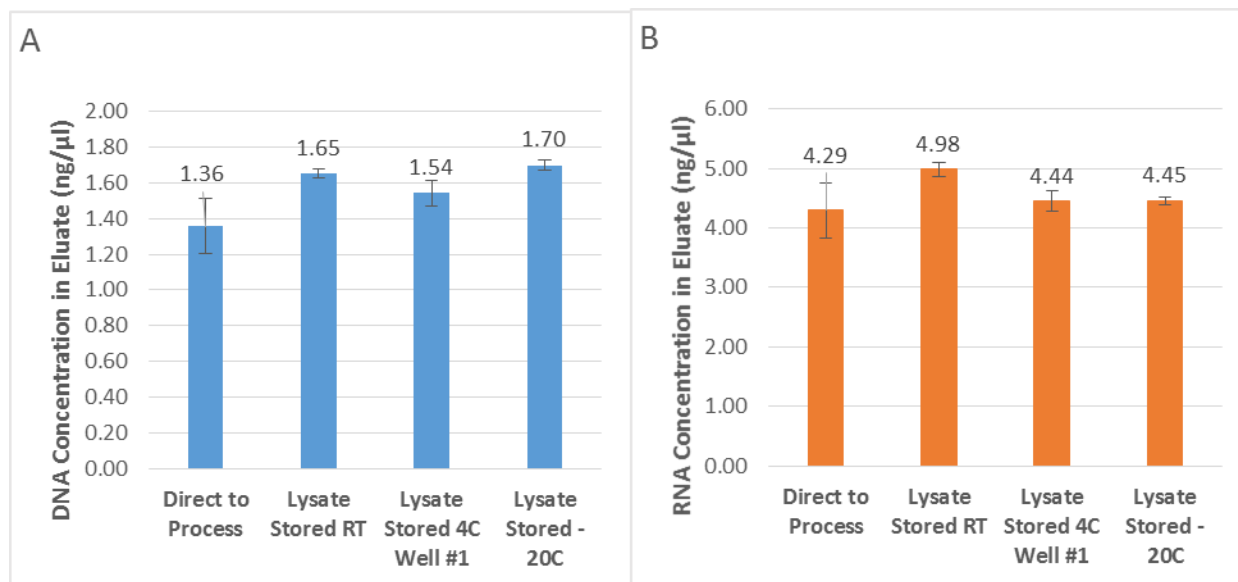


Figure 1. DNA and RNA recovery from FFPE lysates (from SureShot™ Control Samples) stored under different conditions before processing for TNA with the Maxwell® RSC RNA FFPE Kit (Cat.# AS1440) on the Maxwell® RSC Instrument (Cat.# AS4500). After preparing lysates, samples were directly processed, stored at room temperature for 4 hours, stored in well #1 of the FFPE RNA cartridge at 4°C for 4 hours, or frozen at -20°C for 4 hours. The recovery of DNA (panel A) was assessed by qPCR using the GoTaq® qPCR Master Mix (Cat.# A6001) and DNA-specific GAPDH primers and for RNA (panel B) by RT-qPCR using the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and RNA-specific B2M primers. The concentrations of nucleic acid in the eluate were determined based on a standard curve prepared with human genomic DNA (Cat.# G3041) or XpressRef Universal Human Total RNA (Qiagen). Error bars represent the standard deviation for samples purified in triplicate and amplified in duplicate.