Promega

Product Application

Maxwell® RSC FFPE DNA Kit: Alternative Preprocessing Workflows

Isolate amplifiable FFPE DNA using the Maxwell® RSC FFPE DNA Kit following a modified protocol that allows an intermediate overnight storage step.

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

Analyses: qPCR

Sample Type(s): Human FFPE sections

Input: One 10µm section per purification

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**

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC FFPE DNA Kit (Cat.# AS1450)

For overnight hold on a programmable heat/cool block:

- 1 dry block heater set to 56°C
- 1 programmable dry heat/cool block set to 80°C (4 hours) followed by a hold at ≤10°C (e.g., Eppendorf ThermoMixer® R)

For overnight hold at –20°C:

- –20°C freezer storage
- 1 dry block heater set to 56°C
- 1 dry block heater set to 80°C

Protocol:

An overnight hold can be accomplished using either a programmable heat/cool block programmed to perform decrosslinking and then ramp to 4°C for overnight storage, or by storing samples at –20°C following decrosslinking. Both options are described in the protocol below.

1. Preheat one dry heat block to 56°C.
   a. For overnight hold on a programmable heat/cool block, program the second block to hold at 80°C for 4 hours, and then ramp to ≤10°C.
   b. For overnight storage at –20°C, preheat the second block to 80°C.
2. Preprocess samples through Proteinase K digest (56°C) as indicated in the technical manual.
3. Secure tube lids to prevent them from opening when heated, and transfer tubes to 80°C.
   a. For overnight hold on a programmable heat/cool block, tubes can remain overnight on the block at ≤10°C.
   b. For overnight hold at –20°C, transfer tubes to –20°C following the 4-hour incubation.
4. Next day, briefly warm samples to room temperature.
5. Proceed with the standard protocol, starting with RNase A addition.

Results:

Consecutive sections of a human normal colon or lung FFPE samples were interspersed between test groups (n=4 per method). One set was purified as per the standard protocol. The second set was purified using the alternative protocols above. All DNA eluates were amplified using qPCR and DNA concentration quantified in comparison to a standard curve. DNA yield and amplifiability are unaffected using the alternative workflow with an overnight hold at 4°C on a programmable heat/cool block (Figure 1) or with storage at –20°C after decrosslinking (Figure 2).

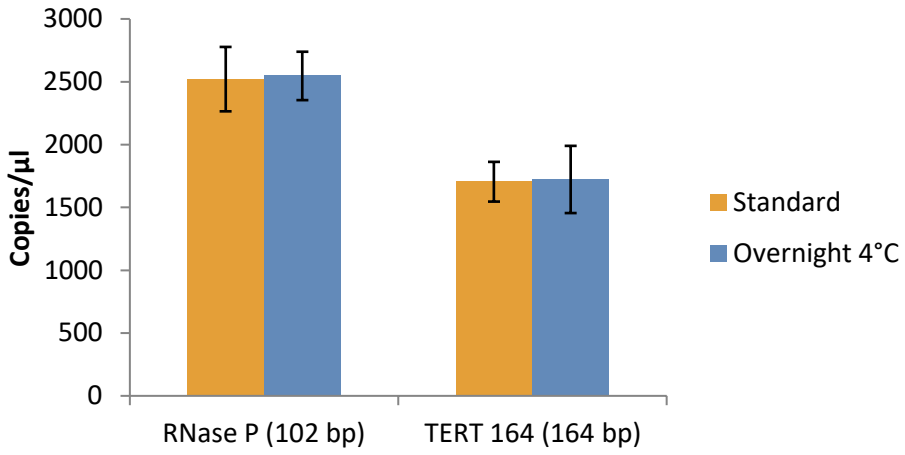

Figure 1. Yield of amplifiable DNA is unaffected using the alternative workflow with an overnight hold at 4°C on a programmable ThermoMixer. Normal human colon FFPE was purified with the Maxwell® RSC FFPE DNA Kit (Cat.# AS1450) using the standard protocol (Standard) or the alternative protocol (Overnight 4°C). DNA eluates were amplified by qPCR to RNase P and TERT and concentrations calculated using a standard curve (mean ± STD, n=4).

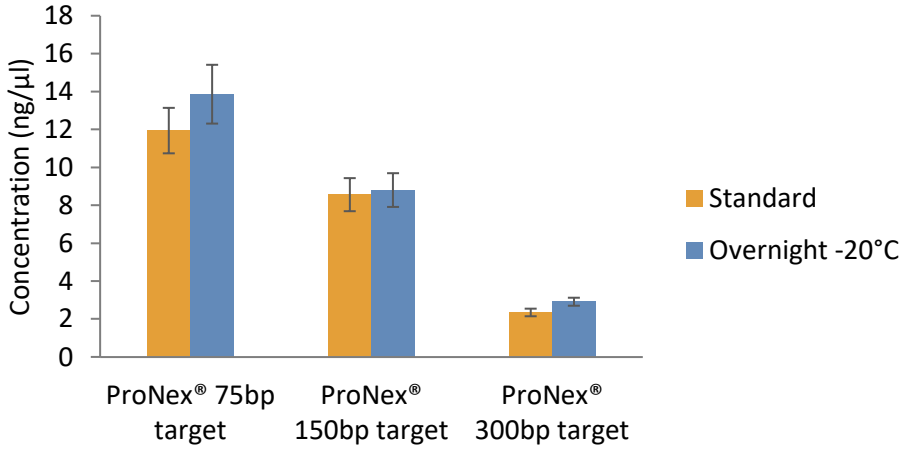

Figure 2. Yield of amplifiable DNA is unaffected using the alternative workflow with sample storage at –20°C after decrosslinking. Normal human lung FFPE was purified with the Maxwell® RSC FFPE DNA Kit (Cat.# AS1450) using the standard protocol (Standard) or the alternative protocol with storage at –20°C for 10 days after decrosslinking (Overnight –20°C). DNA eluates were amplified by qPCR using the ProNex® DNA QC Assay (Cat.# NG1003) with multiplexed targets of 75bp, 150bp and 300bp. Concentrations were calculated using a standard curve (mean ± STD, n=4).