Promega

Product Application

DNA and RNA Isolation from a Single FFPE Sample on the Maxwell® RSC instrument

Purify DNA and RNA from a single FFPE lysate using the Maxwell® RSC Instrument.

Kit: Maxwell® RSC RNA FFPE Kit (Cat.# AS1440) and Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)

Analyses: qPCR and RT-qPCR amplification

Sample Type(s): FFPE tissue sections from lung, breast tumor, and colon tumor tissues.

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.

Further information can be found in Technical Manuals TM436 and TM437 available at: **www.promega.com/protocols**

or by e-mailing technical services at **techserv@promega.com**

Materials Required:

- Maxwell® RSC RNA FFPE Kit (Cat.# AS1440)
- Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat blocks at 56°C and 80°C

Protocol:

1. Add 300µl of mineral oil to an FFPE section in a 1.5ml microcentrifuge tube. Vortex for 10 seconds.
2. Heat the samples at 80°C for 2 minutes.
3. Prepare a lysis master mix of 224µl of Lysis Buffer, 25µl of Proteinase K and 1µl of Blue Dye per sample.
4. Add 250µl of the lysis master mix to each sample tube, and vortex for 5 seconds.
5. Centrifuge samples at 10,000 *x g* for 30 seconds. Mix sample by pipetting if there is a pellet present.
6. Incubate the sample tubes at 56°C for 30 minutes.
7. Incubate the sample tubes at 80°C for 2 hours.
8. Remove samples and cool to room temperature for 15 minutes.
9. Centrifuge samples at maximum speed for 2 minutes.
10. Split blue, aqueous phase into two microfuge tubes (~120µl each).
    a. RNase Treatment (Tube #1 – DNA sample)
        i. Add **5µl of RNase A** to the aqueous phase lysate (blue layer), mix by pipetting.
        ii. Incubate at room temperature for 5 minutes.
    b. DNase Treatment (Tube #2 – RNA sample)
        i. Create DNase cocktail with **13µl of $MnCl_2$, 7µl of DNase Buffer, and 5µl of DNase 1** per lysate.
        ii. Add **25µl of the cocktail** to the aqueous phase lysate (blue layer) and mix by pipetting.
        iii. Incubate at room temperature for 15 minutes.
11. Set up two Maxwell® FFPE cartridges (one from the DNA FFPE kit and the other from the RNA FFPE kit). Transfer the lysate from Tube #1 (contains DNA) into well #1 of a Maxwell® RSC DNA FFPE cartridge and transfer lysate from Tube #2 (contains RNA) into well #1 of a Maxwell® RSC RNA FFPE cartridge.
12. Elute in 50µl of Nuclease-Free Water for each kit.
13. Start the method: either the DNA FFPE or RNA FFPE method on the Maxwell® RSC instrument, depending on nucleic acid species.

Results

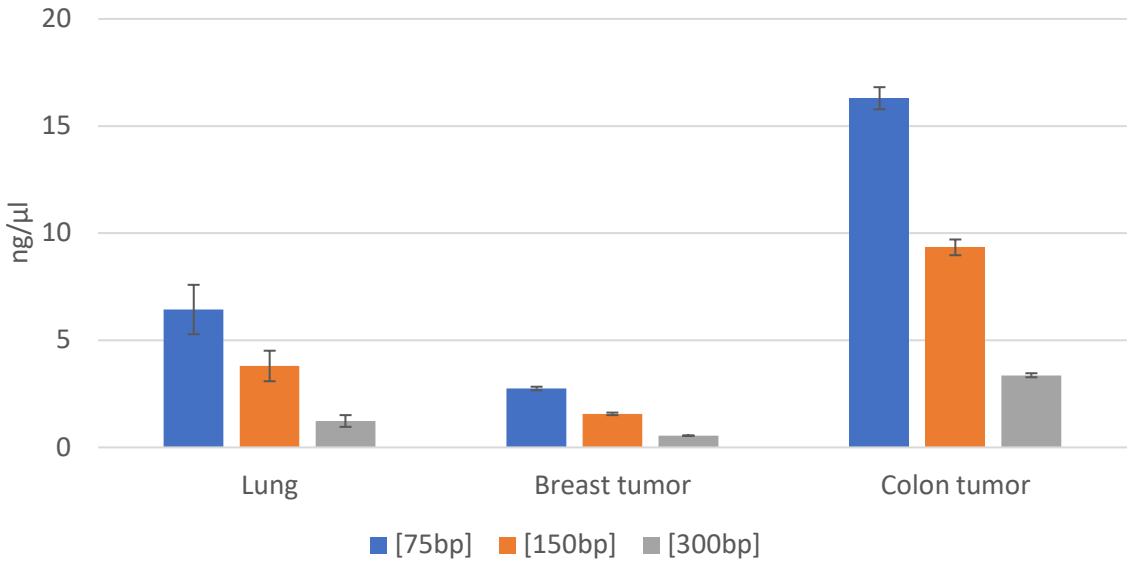

Figure 1: Concentration of DNA isolated from FFPE sections (three tissue types). DNA concentrations were measured by performing multiplex qPCR with a 75bp, a 150bp and a 300bp target and concentrations were determined relative to a human gDNA standard curve (amplification with the kit ProNex® DNA QC Assay ABI 7500/7500FAST (Cat.# NG1002). Data are shown as mean ± standard deviation of n=3.

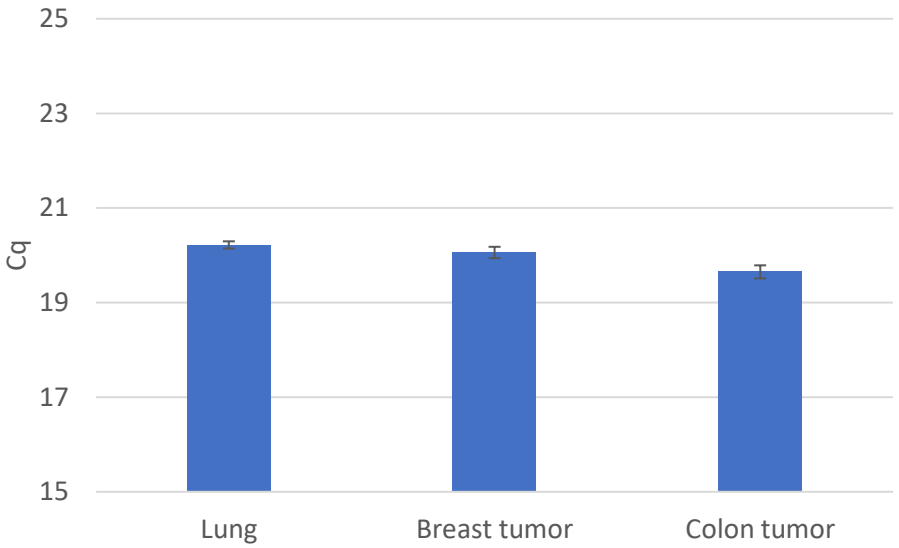

Figure 2. Cq values from RT-qPCR with RNA isolated from FFPE sections (three tissue types). RNA was amplified with RNA-specific Beta-2-Microglobulin primers and GoTaq® 1-Step RT-qPCR System (Cat.# A6020). Data are shown as mean ± standard deviationof n=3