

## Purification of DNA and RNA from a Single FFPE Sample with ReliaPrep™ FFPE kits

*Manual isolation of DNA and RNA from a single or multiple FFPE lysates.*

**Kit:** ReliaPrep™ FFPE gDNA Miniprep System (Cat.# A2352)  
ReliaPrep™ FFPE Total RNA Miniprep System (Cat.# Z1002)

**Analyses:** qPCR, RT-qPCR

**Sample Type(s):** FFPE tissue sections

**Input:** 5-10µM sections.

**Materials Required:**

- ReliaPrep™ FFPE gDNA MiniPrep System (Cat.# A2352)
- ReliaPrep™ FFPE Total RNA MiniPrep System (Cat.# Z1002)
- 95-100% Ethanol
- 100% Isopropanol
- Heat block set to 80°C
- Heat block set to 56°C

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.

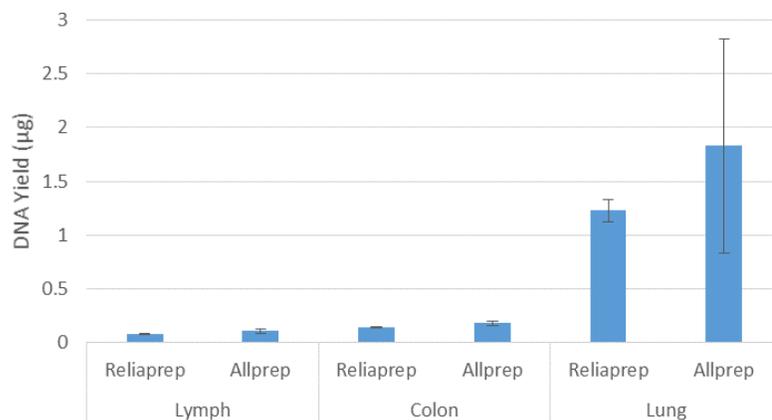
For further information, see Technical Manual TM352 and TM353, available at: [www.promega.com/protocols](http://www.promega.com/protocols) or for further information, please contact [techserv@promega.com](mailto:techserv@promega.com)

**Protocol:**

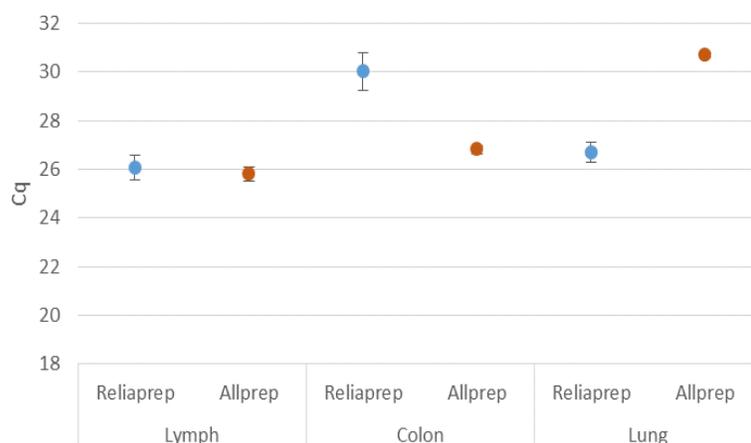
1. Add 300µl Mineral Oil to FFPE section in a 1.5ml microcentrifuge tube. Vortex for 10 seconds.
2. Heat the samples at 80°C for 1 minutes, vortex to mix.
3. Add 400µl of Lysis Buffer.
4. Centrifuge samples at 10,000 x g for 30 seconds.
5. Add 40µl Proteinase K to bottom aqueous layer and mix by pipetting.
6. Transfer the sample tubes to 56°C heat block and incubate for 60 minutes.
7. Transfer the sample tubes to 80°C heat block and incubate for 60 minutes.
8. Remove samples and cool to room temperature for 15 minutes.
9. Centrifuge samples at max speed for 2 minutes. Split aqueous phase into two tubes (~200µl each).
10. Add Lysis Buffer to bring the total volume of each to 220µl.
  - a. RNase Treatment (lysate # 1)
    - i. Add 10µl RNase A to the lysate, mix with pipet
    - ii. Incubate at room temperature for 5 minutes
  - b. DNase Treatment (lysate #2)
    - i. Create DNase cocktail by mixing 13µl MnCl<sub>2</sub>, 7µl DNase Buffer, and 10µl DNase I per reaction
    - ii. Add 30µl of the cocktail to the lysate and mix with pipet
    - iii. Incubate at room temperature for 15 minutes
11. Continue with protocols from each kit's technical manual TM352 (FFPE gDNA Miniprep) and TM353 (FFPE Total RNA Miniprep) for washing and eluting.

## Results:

A single FFPE section (three tissue types) was processed using ReliaPrep™ FFPE gDNA and ReliaPrep™ Total RNA Miniprep Systems with the protocol described above or with the AllPrep® DNA/RNA FFPE Kit (Qiagen). Amplifiable DNA concentrations were measured by qPCR with a 75 base pair target (Figure 1). Cq values from RT-qPCR amplification of the isolated RNA, using RNA specific primers are shown in Figure 2.



**Figure 1. Yield of DNA isolated from FFPE tissues using the ReliaPrep™ FFPE gDNA Miniprep System (Cat.# A2352) and the AllPrep® DNA/RNA FFPE Kit (Qiagen).** DNA concentrations were measured by performing qPCR with a 75 base pair target and then using linear regression from a human DNA standard curve. Yields were calculated by multiplying the concentration by the elution volume. Shown is the average  $\pm$  standard deviation of four separate extractions for each condition (N=4).



**Figure 2. Cq values from RT-qPCR of RNA isolated from FFPE tissues using the ReliaPrep™ FFPE Total RNA Miniprep System (Cat.# Z1002) and the AllPrep® DNA/RNA FFPE Kit (Qiagen).** RNA was amplified with RNA specific Beta 2 Microglobulin primers. Shown is the average  $\pm$  standard deviation of four separate extractions for each condition (N=4).