Promega

Product Application

Isolation of DNA and RNA from a single FFPE sample

Isolate amplifiable DNA and RNA from a single FFPE tissue sample by splitting the lysate between two ReliaPrep™ Purification kits.

Kits: ReliaPrep™ FFPE gDNA Miniprep System and ReliaPrep™ FFPE Total RNA Miniprep System

Analyses: Quantitation using GoTaq® qPCR and RT-qPCR, and QuantiFluor® ONE dsDNA and QuantiFluor® RNA Systems

Sample Type(s): FFPE Tissue

Input: 1 FFPE curl per nucleic acid isolation

This protocol was developed by Promega Applications Scientists and is intended for research use only.

The user is responsible for determining its suitability in the user's application.

Further information can be found in Technical Manuals #TM352 and #TM353, available at: **www.promega.com/protocols**

Materials Required:

- ReliaPrep™ FFPE gDNA Miniprep System (Cat. #A2352)
- ReliaPrep™ FFPE Total RNA Miniprep System (Cat. #Z1002)
- Heat block – 56°C and 80°C
- Microcentrifuge

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 200µl of Lysis Buffer.
4. Centrifuge samples at 10,000 *x g* for 30 seconds.
5. Add 20µl 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.
10. Split aqueous phase into two microfuge tubes (~100µl each).
11. 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 $MnCl_2$, 7µl DNase Buffer, and 10µl DNase 1 per reaction

ii. Add 30µl of the cocktail to the lysate and mix with pipet

iii. Incubate at room temperature for 15 minutes

12. Continue with protocols from each kit's technical manual for washing and eluting.

Results: The above protocol was tested with human colon FFPE tissue.

Table 1. Nucleic acid yields determined by QuantiFluor® dye systems: 2µl of each eluate was analyzed on the Quantus Fluorometer (E6150) using the QuantiFluor® ONE dsDNA System (E4871) and QuantiFluor® RNA System (E3310).

	DNA (ng)	RNA (ng)
Colon Rep. 1	1570	1698
Colon Rep. 2	1551	1971
Colon Rep. 3	1489	1773
Average	1537	1814
Deviation	42	141

Table 2. Concentrations of amplifiable DNA and RNA determined by qPCR and RT-qPCR, respectively. GoTaq® qPCR Master Mix (A6001) with DNA-specific GAPDH primers (100bp and 300bp amplicon sizes) and Human Genomic DNA (G3041) were used for absolute quantitation of DNA in the eluates. GoTaq® Probe 1-Step RT-qPCR System (A6120) with RNA-specific B2M primers and Human Reference Total RNA (Stratagene, 750500) were used for absolute quantitation of RNA in the eluates.

	DNA (ng) 100bp	DNA (ng) 300bp	RNA (ng)
Colon Rep.1	489	119	9
Colon Rep.2	514	136	9
Colon Rep.3	493	129	11
Average	499	128	10
Deviation	13	8	1