MP Biomedicals

Case study - *Skin Tissue*

Health

Optimized Methodology for Sequential Extraction of RNA and Protein from Small Human Skin Biopsies.

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J Invest Dermatol. 2007. Vol 127.

Introduction

Skin tissue, although easily accessible, is difficult to process owing to its natural resistance to mechanical shearing and high levels of RNases and proteases. Currently, these complications result in degraded RNA samples with variable yield.

We have developed a method of sequential extraction of high quality RNA and protein from a single 3 mm full thickness skin punch biopsy.

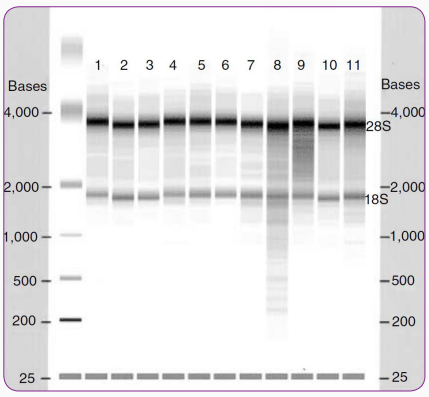
Overview

- **Keywords:** Tissue biopsy, clinical samples, RNA extraction, protein isolation
- **Aim of the study:** Optimization of RNA and Protein extraction from skin tissue
- **Application:** Western blot & quality RNA analysis
- **Sample name:** Tissue biopsy
- **Sample type:** Human skin biopsies from a 3 mm punch
- **Material:** FastPrep-24™ instrument, Lysing matrix D tubes
- **Buffer:** Guanidine thiocyanate lysis buffer

Protocol and Parameters

1. Add the 19 mg of skin sample to a Lysing Matrix D tube
2. Add 1 ml of a guanidine thiocyanate lysis buffer (5.1M guanidine thiocyanate, 50 mM sodium citrate, 50 mM EDTA, 0.5% β-mercaptoethanol).
3. Homogenize in the FastPrep-24™ instrument for 3 x 40 seconds at a speed setting of 6.0. Place the tubes on ice for 5 mins between each run.
4. Centrifuge at 14,000 x g for 5-10 mins to pellet debris
5. Proceed with the RNA and protein extraction protocol

Results



High quality RNA isolation with FastPrep-24™ homogenizer

RNA 2100 Bioanalyzer analysis of FastPrep® samples.

The RNA was run on an Agilent 2100 Bioanalyzer *(Agilent, Palo Alto, CA)* using the RNA 6000 Pico LabChip kit to determine the quality of the samples. The 28S and 18S ribosomal bands show a greater than 2:1 ratio and the calculated RNA ribosomal integrity numbers of the samples ranged from 8.4 to 8.9 verifying high quality RNA. Gel images for 11 RNA samples.

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Results

	RNA average quantity per biopsy (μg)	RNA average 260/280 ratio	Protein average quantity per biopsy (μg)
FastPrep bead-beater	1.4 (±0.4 μg)	2.0 (±0.05)	170 (±50 μg)
Homogenizer	0.8 (±0.4 μg)	1.8 (±0.11)	90 (±40 μg)

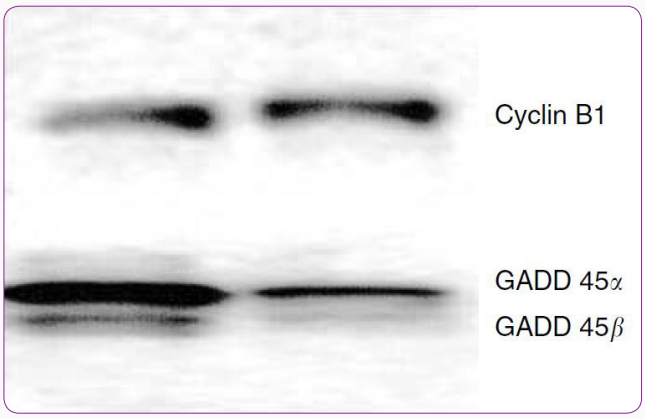
For each method of tissue disruption, the quantity and quality of RNA (as an OD260/280 ratio), and the quantity of protein is shown. The RNA was quantified using the Nanodrop spectrophotometer and the protein content was determined using a Bradford-based assay. For RNA, an OD260/280 of 2.0 is optimal. All quantities are ± SD.

Higher RNA & protein yield obtained with FastPrep-24™ homogenizer

RNA and protein quantification. For each method of tissue disruption, the quantity and quality of RNA (asan OD260/280 ratio), and the quantity of protein is shown. The RNA was quantified using the Nanodrop spectrophotometer and the protein content was determined using a Bradford-based assay. For RNA, an OD260/280 of 2.0 is optimal.

Quality assessment of extracted protein

Western blots using biopsy sample protein. Approximately 10–15 mg of protein from two different biopsy samples processed with the FastPrep 120 *(Q-BIOgene, Irvine, CA)* were used to determine the quality of Western blotting. The top panel was probed with mouse anti-cyclin B1 and the bottom panel is mouse anti-GADD 45. The GADD 45 antibody used *(Santa Cruz Biotechnology Inc., Santa Cruz, CA)* recognizes both the alpha and beta subunits of the protein.



Conclusion

- Sample variability and exposure to exogenous contamination were reduced using the FastPrep® bead beating, this instrument allowed processing up to 24 samples very quickly.
- This method yields 1–2 mg of RNA and 150 mg of protein, which is usable in many sensitive downstream applications including microarray, quantitative real-time PCR, two-dimensional gel electrophoresis and Western blot analysis.

Successful sample preparation using the MP Biomedicals FastPrep® product line has been highlighted in thousands of scientific articles. To access articles and other materials, visit www.mpbio.com/FastPrepLibrary.

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