APPLICATION OVERVIEW TISSUE

Sample Overview

Animal and human tissues can be the toughest samples to isolate high-quality DNA, RNA, and proteins. Using the **FastDNA Spin Kit** and **FastRNA Pro Green Kit** in combination with the **FastPrep** instrument, full homogenization of any sample including bone and tumors, and more elastic samples like skin, is achieved within a few seconds. This method saves hours of work during sample preparation and provides high yields of DNA, RNA, and proteins.

Effective, efficient sample preparation is critical to successful downstream results.

RNA and Protein Extraction From Skin Tissue

The FastPrep and associated matrices have demonstrated successful lysis and dual extraction of RNA and proteins from skin tissue in three runs of 40 seconds each.

Materials

- FastPrep instrument
- Lysing Matrix D tubes
- Sample: Human skin biopsies from a 3-mm punch, weighing only 19 mg on average

Protocol and Parameters

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

Results

- The average yield of 1.4 µg RNA obtained with the FastPrep System was 57% higher than yields obtained with the Polytron (Figure 1).
- The average yield of 170 µg protein obtained with the FastPrep System was 53% higher than yields obtained with the Polytron (Figure 1).

Results continued on back

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

Figure 1. RNA and protein quantitation 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.

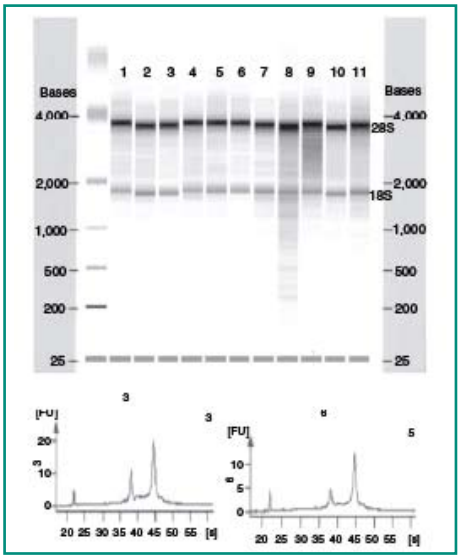

Figure 2. The RNA was run on an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, Calif.) using the RNA 6000 Pico LabChip kit to determine the quality of the samples. The 28S and 18S ribosomal bands show a greater than 2:1 ratio, and the calculated RNA ribosomal integrity numbers of the samples ranged from 8.4 to 8.9, verifying high-quality RNA. Shown above are the gel images for 11 RNA samples and below are two representative electrophoretic graphs showing the RNA peaks.

APPLICATION OVERVIEW TISSUE

- To verify the high-quality nature of the RNA, samples were analysed with the Agilent 2100 Bioanalyzer. samples had ribosomal integrity number numbers of between 8.4 and 8.9, which is consistent with high-quality RNA (Figure 2).
- The quality of extracted proteins was assessed by two-dimensional gel and Western blot analysis. There was distinct spot resolution and sufficient protein isolated from single biopsy to produce five to six two-dimensional gels. For Western blotting, a primary antibody against GADD-45 was used to probe the membrane. GADD-45 antibody detects both the alpha and beta portions of the protein, although it is more sensitive for the alpha portion.

Reference: Berglund SR, Schwietert CW, Jones AA, Stern LR, Lehmann J, Goldberg Z. Optimized methodology for sequential extraction of RNA and protein from small human skin biopsies. J Invest Dermatol. 2007;127:349-53.

Product Overview

The **FastDNA Spin Kit** is used with the **FastPrep** instrument to lyse and subsequently isolate DNA from up to 200 mg of almost any sample in less than 30 minutes.

The **FastRNA Pro Green Kit** is designed to efficiently isolate total RNA from any type of plant and animal tissue or cultured cells.

Typical Tissue Settings

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep Speed	FastPrep Time
Human	Lung	50 mg	D	6.0	4 x 30 sec
Human	Breast	80 mg	D	6.0	2 x 30 sec
Human	Kidney	50 mg	D	6.0	40 sec
Human	Thyroid Tumors	100 mg	A	6.0	3 x 30 sec
Mouse	Eye	10 mg	D	6.0	4 x 30 sec
Mouse	Heart	70 mg	D	6.0	4 x 30 sec
Mouse	Kidney	50 mg	D	6.0	40 sec
Mouse	Femur	40 mg	A	6.0	4 x 30 sec
Mouse	Leg Muscle	50 mg	D	6.0	40 sec
Mouse	Intestine	50 mg	D	6.0	40 sec
Mouse	Ear	45 mg	D	6.0	4 x 30 sec
Mouse	Tail	100 mg	A	6.0	4 x 30 sec
Mouse	Spleen	70 mg	D	6.0	40 sec
Mouse	Lung	50 mg	D	6.0	40 sec
Mouse	Liver	50 mg	D	6.0	40 sec
Mouse	Brain	50 mg	D	6.0	40 sec
Mouse	Pancreatic Cells (bHC9)	10^7 cells	D	6.0	40 sec

References

Successful sample preparation using the MP Biomedicals product line has been highlighted in hundreds of scientific articles. **To access articles and other educational materials, visit www.mpSamplePrep.com.**