

RNA/DNA/Protein Purification Plus Micro Kit

Norgen's RNA/DNA/Protein Purification Plus Micro Kit provides a rapid method for the isolation and purification of total RNA, genomic DNA and proteins sequentially from a single sample of cultured animal cells, small tissue samples, microdissected samples including laser-capture microdissection (LCM), blood, fungi or plants. The total RNA, genomic DNA and proteins are all column purified in less than 30 minutes. The RNA and DNA can be eluted in as little as 20 μ L while the protein can be eluted in as little as 50 μL. This kit is ideal for researchers who are interested in studying the genome, proteome and transcriptome of a single sample, such as for studies of microRNA profiling, gene expression including gene silencing experiments or mRNA knockdowns, studies involving biomarker discovery, and for characterization of cultured cell lines. This kit is especially useful for researchers who are isolating macromolecules from precious, difficult to obtain or small samples as it eliminates the need to fractionate the sample. Analysis will be more reliable since the RNA, DNA and proteins are derived from the same sample, thereby eliminating inconsistent results. The purified macromolecules are of the highest purity and can be used in a number of different downstream applications.



Purification is based on spin column chromatography. The sample is first lysed, and the DNA is then captured and purified on a gDNA Purification Micro Column. The flowthrough of the DNA purification step is then loaded onto a RNA/Protein Purification Micro Column. Total RNA, including microRNAs, will bind to the column while the proteins are removed in the flowthrough. The bound RNA is then washed and eluted from the column. The same RNA/Protein Purification Micro Column is then used to purify the proteins that are present in the RNA binding flowthrough. Alternatively, the kit is provided with a specially formulated loading dye that allows the proteins present in the flowthrough to be loaded directly onto an SDS-PAGE gel. Norgen's kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array analysis. The genomic DNA is also of the highest quality, and can be used in various applications including PCR reactions, sequencing, Southern blotting and SNP analysis. The purified proteins can also be used in a number of different downstream applications, such as SDS-PAGE analysis and Western blots.

RNA/DNA/Protein Purification Plus Micro Kit Benefits			
Complete column purification	The RNA, DNA and proteins are all column purified.		
Small elution volumes	Small elution volume results in a higher concentration of eluted RNA/ DNA/Protein		
Reduce variability	RNA, DNA and proteins are isolated from a single sample with no splitting of the lysate, thus reducing inconsistent results and variability.		
Isolate from small samples	Sequential isolation of RNA, DNA and protein from a single sample. Ide- al for precious, difficult to obtain or small samples such as biopsy materi- al or single foci from cell cultures.		
Rapid procedure	Isolate total RNA, genomic DNA and total proteins from a single sample in < 30 minutes.		
lsolate a diversity of RNA species	All sizes of RNA are isolated, from large mRNA down to microRNA		



RNA/DNA/Protein Purification Plus Micro Kit

Kit Specifications			
Column Binding Capacity (RNA)	35 µg	Average Yield:	
Column Binding Capacity (DNA)	10 µg	HeLa Cells (5 x 105 cells)	7.5 μg RNA
Column Binding Capacity (Protein)	100 µg	HeLa Cells (5 x 10⁵ cells)	1-2 μg DNA
Size of RNA Purified	All sizes	HeLa Cells (5 x 10 ⁵ cells)	70 µg protein
Size of DNA Purified	> 30 kbp	Time to Complete 10 Purifications	30 minutes

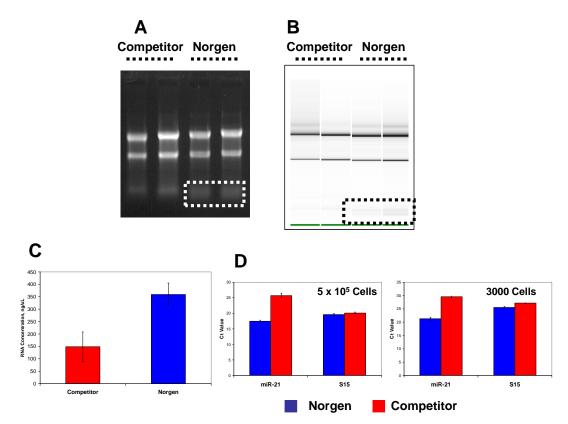


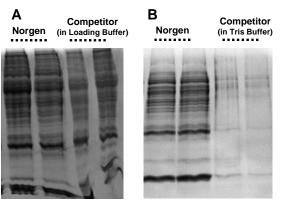
Figure 1. Recovery of True Total RNA including microRNA from HeLa Cells

Panel A is a 1X MOPS 1% agarose gel showing the RNA that was isolated from 2 different samples of ~500,000 HeLa cells using either Norgen's RNA/DNA/Protein Purification Plus Micro Kit or a competitor's multiple-analyte purification kit. Both kits isolated large RNA (represented by 28S and 18S rRNA) with high integrity but Norgen's RNA/DNA/Protein Purification Plus Micro Kit provided the added benefit of recovering small RNA without any additional protocols. Panel B is a result from a bioanalyzer resolution of the eluted RNA. Similar to the agarose gel, Norgen's RNA/DNA/Protein Purification Plus Micro Kit showed the added benefit of recovery was also demonstrated by gene-specific RT-qPCR. Two microliters of RNA isolated from both 500,000 and 3,000 HeLa cells were used in RT-qPCR reactions for human S15 (for Large RNA) and miR-21 (for microRNA) genes. The RNA isolated by Norgen's RNA/DNA/Protein Purification Plus Micro Kit showed similar or better (lower) Ct value than RNA isolated by the competitor's kit for the large RNA. More importantly, Norgen's RNA/DNA/Protein Purification Plus Micro Kit showed superior recovery of small RNA isolated by the miR-21 RT-qPCR (Panel D)

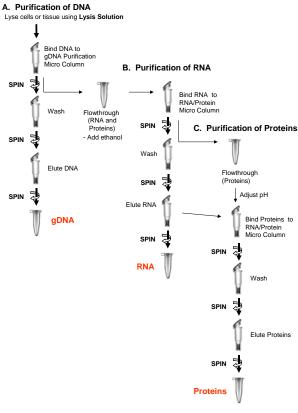


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Figure 2. High Quality Total Proteins Eluted in Mass Spec-Compatible Buffer.



RNA/DNA/Protein Purification Plus Micro Kit Procedure



Protein fractions (from hamster liver) isolated by Norgen's RNA/DNA/Protein Purification Plus Micro Kit and a competitor's kit were resolved on a 12% SDS-PAGE protein gel. Panel A shows that when the competitor's precipitated protein fraction was resuspended in a provided SDS-PAGE loading buffer, the protein recovery was similar among the two kits. Panel B shows that when the same precipitated protein fraction from the competitor's kit was resuspended in a Tris-based buffer containing no detergent or denaturant, the protein recovery became drastically reduced. In contrary, Norgen's RNA/DNA/ Protein Purification Plus Micro Kit purified proteins by column and the eluted proteins are already in a buffer compatible with most downstream applications including mass spectrophotometry as well as standard protein quantification methods (including Bradford assays).

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 96-100% ethanol
- β-mercaptoethanol (optional)
- RNase-free DNase I (optional)
- RNase-free PBS (Animal Cells)
- Liquid nitrogen, mortar and pestle (Tissue, Fungi, Plant)
- Sterile fine forceps, Water bath at 42°C (LCM)

Storage Conditions

The Protein Loading Dye should be stored at -20°C upon arrival. All other solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers

Shipping Conditions

The RNA/DNA/Protein Purification Plus Micro Kit is shipped at room temperature.

Cat #	Description	Quantity
51600	RNA/DNA/Protein Purification Plus Micro Kit	50 preps

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