

Sequential Purification of RNA, DNA and Protein from a Single Sample using Norgen's RNA/DNA/Protein Purification Kit and Comparison to a Market Competitor

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INTRODUCTION

Norgen's RNA/DNA/Protein Purification 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, blood, bacteria, yeast, fungi or plants. The kit streamlines sample preparation by enabling the simultaneous purification of nucleic acids and proteins from the same cell or tissue sample. As there is no need to split the sample prior to purification, the recovery of all analytes is maximized. In addition, no organic solvents such as phenol or acetone are required for the procedure.

The total RNA, genomic DNA and proteins are all column purified in less than 35 minutes using Norgen's proprietary resin as the separation matrix (**Figure 1**). The kit is ideal for research on the study of the genome, proteome and transcriptome of a single sample, such as for gene expression studies, including gene silencing or mRNA knockdowns, biomarker discovery and for characterization of cultured cell lines. Norgen's RNA/DNA/Protein Purification Kit is especially useful for researchers who are isolating macromolecules from precious, difficult to obtain or small samples such as biopsy materials or single foci from cell cultures, as it eliminates the need to fractionate the sample. Norgen's RNA/DNA/Protein Purification Kit standardizes sample preparation for systems biology. Since the DNA, RNA and proteins are all prepared from the same source it eliminates the variation inherent in preparing these analytes from different samples. Furthermore, analyses will be more reliable since the RNA, DNA and proteins are derived from a single sample in less than 30 minutes. The purified macromolecules are of the highest purity and can be used in a number of different downstream applications.

In this application note, Norgen's RNA/DNA/Protein Purification Kit is used to isolate total RNA, DNA and proteins from a single sample of HeLa cells. Kit performance is compared to the leading market competitor's multi-analyte kit in terms of ease of use, sample preparation time and quality of purified analytes. Furthermore, downstream applications of RT-PCR for RNA and Western blot for protein are performed, indicating the purity and quality of each of the purified biomolecules.

METHODS AND MATERIALS

Sequential RNA-DNA-Protein Isolation

RNA, DNA and proteins were isolated from 5×10^5 HeLa cells using Norgen's RNA/DNA/Protein Purification Kit as per the provided protocol (**Figure 1**). Briefly, the cells were pelleted by centrifugation, the media removed and the pellet washed once with PBS. Lysis solution was then added to the cell pellet, followed by the addition of the ethanol. The lysate was then loaded onto a provided column, and the flowthrough that passed through the column was collected for subsequent protein purification. The column was then washed twice with 400 μ L of the RNA Wash Solution, and the purified RNA was then eluted with 50 μ L of RNA Elution Solution. The column was then washed once more with the gDNA Wash Solution. This was followed by the elution of purified gDNA using the gDNA Elution Buffer. Next, the spin column was regenerated and activated for protein purification using 500 μ L of Protein Column Regeneration Buffer and 500 μ L of Protein Column Activation and Wash Buffer, respectively. The flowthrough from the first column loading contained the proteins and was then pH adjusted using the Protein pH Binding Buffer. The pH-adjusted lysate was re-loaded onto the column in order to bind the proteins. The column was washed once with 500 μ L of Protein Column Activation and Wash Buffer, and finally the purified proteins were eluted with 100 μ L of Protein Elution Buffer and neutralized with 9.3 μ L of Protein Neutralizer.

At the same time, RNA and proteins were purified from HeLa cells (5×10^5) using the leading market competitor's RNA/Protein purification kit according to the manufacturer's methods. Purified RNA and protein were then used in comparative experiments.

RNA Gel Electrophoresis

The purified RNA was resolved on a 1X MOPS, 1.2% formaldehyde-agarose gel for visual inspection. A total of 7.5 μ L of each 50 μ L elution was run on the gel.

DNA Gel Electrophoresis

The purified genomic DNA was resolved on 1X TAE, 1% agarose gels for visual inspection. A 15 μ L aliquot of each 100 μ L elution was run on the gel.

Nucleic Acid Quantification and Purity

The quantification and purity of both total RNA and genomic DNA samples was determined spectrophotometrically.

Protein Gel Electrophoresis

The purified proteins were resolved on 10% SDS-PAGE gels, followed by Coomassie Blue Staining for visual inspection. Typically, 10 μ L of each 100 μ L elution was run on the gel. Please note that alternatively, the proteins from the initial lysate may also be directly loaded onto an SDS-PAGE gel (without column purification) using the provided Protein Loading Dye.

Protein quantification

Protein quantification was determined by the Bradford Assay according to standard protocols.

RT-qPCR Assay

RNA purified from HeLa cells was used as the template in an RT-qPCR reaction, using primers specific for the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (1).

Capillary Electrophoresis

The purified RNAs were loaded onto an Agilent® RNA Nano 6000 chip and resolved on an Agilent® 2100 BioAnalyzer according to the manufacturer's instructions.

Western Immunoblotting

The purified proteins were resolved by SDS-PAGE as described above and transferred to a PVDF membrane. Western blotting was performed using an anti-GAPDH Antibody (Competitor).

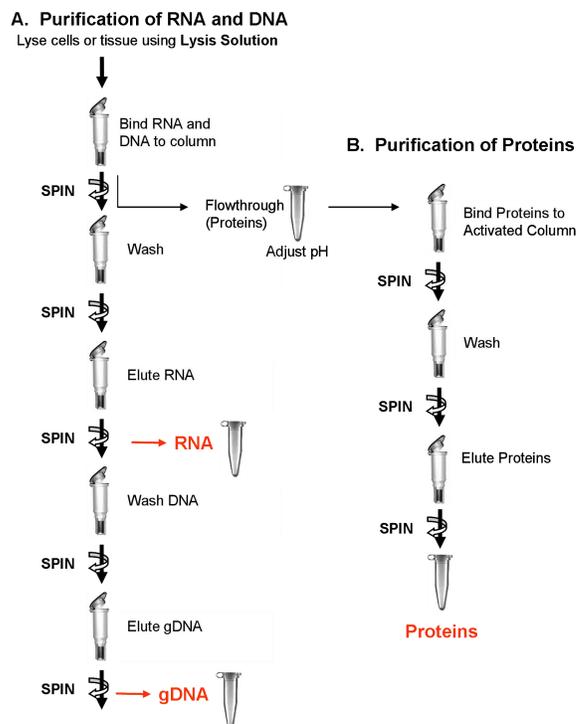


Figure 1. Procedure flowchart for the simultaneous purification of RNA, DNA and proteins from a single sample using Norgen's RNA/DNA/Protein Purification Kit.

RESULTS AND DISCUSSION

RNA, DNA and proteins were sequentially isolated from 5×10^5 HeLa cells using Norgen's RNA/DNA/Protein Purification Kit as per the provided protocol. The protocol was performed in triplicate and the entire protocol was completed in 35 minutes. At the same time RNA and proteins were isolated from the same number of HeLa cells using the leading market competitor's kit. It must be noted that the competitor's kit does not allow for the purification of genomic DNA, and unlike Norgen's kit the sample must be split prior to purification reactions.

Once the nucleic acids were isolated, they were run on agarose gels for visual inspection (**Figure 2**), while the purified proteins were resolved and visualized via SDS-PAGE (**Figure 3**).

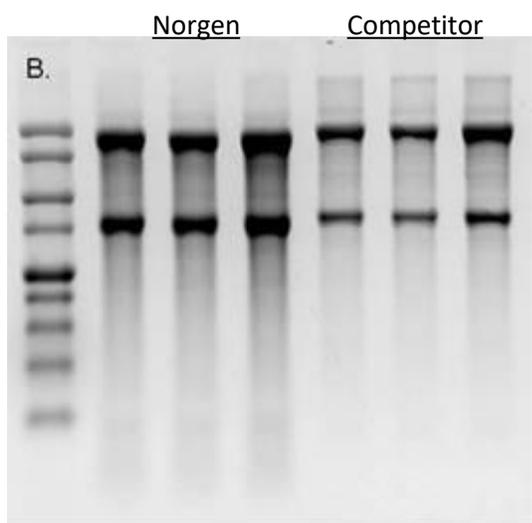


Figure 2. Genomic DNA and Total RNA Isolated from 5 x 10⁵ HeLa cells. Panel A is a 1% agarose gel showing the genomic DNA isolated using Norgen's kit from 3 different samples of HeLa cells. The first lane contains Norgen's UltraRanger DNA Ladder with subsequent lanes containing 15 μ L of each of the 100 μ L elutions. **Note:** The competitor's kit did not offer genomic DNA purification. Panel B is a 1X MOPS, 1.2% formaldehyde-agarose gel showing the RNA that was isolated from the same three samples of HeLa cells using Norgen's kit and the competitor's kit. The first lane contains Norgen's 1Kb RNA Ladder, the next three lanes contain 7.5 μ L of the 50 μ L elutions purified using Norgen's kit, and the last three lanes on the gel contain 7.5 μ L the RNA purified using the competitor's RNA/protein purification kit.

Table 1. Comparison of RNA Quality and Quantity by Spectrophotometry.

| Sample Preparation Kit | RNA Concentration (μ g/ μ L) | A260:A280 | A260:A230 |
|------------------------|---------------------------------------|-----------|-----------|
| Norgen | 0.24 | 2.10 | 2.26 |
| Competitor | 0.09 | 2.11 | 2.78 |

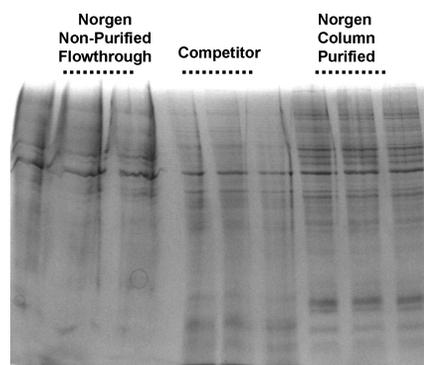


Figure 3. Purified Proteins Isolated from 5 x 10⁵ HeLa Cells. Proteins purified using the Norgen kit or the competitor kit were isolated in triplicate and run on a 10% SDS-PAGE gel. Also shown are the non-purified proteins from the flowthrough using Norgen's kit.

Table 2. Quantification of Protein by the Bradford Assay.

| Sample preparation kit | Protein concentration (μ g/ μ L) |
|------------------------|---|
| Norgen | 0.233 |
| Competitor | 0.503 |
| Norgen purified | 0.611 |

As can be seen in **Figure 2** and **Figure 3**, each of the biomolecules purified was of a high quality. The genomic DNA isolated using Norgen's kit was of a high integrity and purity, with no observable signs of RNA contamination (**Figure 2, Panel A**). Similarly, the RNA isolated was of a

high purity, with no signs of degradation or gDNA contamination (**Figure 2, Panel B**). The Norgen-purified RNA contained the entire spectrum of species, including intact 28S and 18S rRNAs, mRNAs and small RNAs (<200 nt), while the competitor failed to isolate the small RNA species. Also, due to the fact that samples are split prior to processing with the competitor's kit, a lower RNA yield was observed on the gel for samples purified using the kit.

Similar to what was observed on agarose gels, RNA purified using Norgen's kit had a much higher yield than its competitor based on spectrophotometric analyses (**Table 1**). This is due to the fact that unlike its competitor there is no sample splitting prior to purification with Norgen's kit. In addition to high yield, the Norgen purified RNA had good A260:A280 and A260:A230 ratios. This is especially important for downstream applications which demand that purified RNA be of a high quality. Organic contaminants, such as phenol and other aromatic compounds, TRIzol and some additional reagents used in RNA extractions absorb light of a 230 nm wavelength. Samples with a low 260/230 ratio (below 1.8) have a significant presence of these organic contaminants that may interfere with other downstream processes such as RT-qPCR experiments, lowering their efficiency. In order to foster the success of microarray and gene expression experiments, it is recommended to only use samples with a 260/230 ratio greater than 1.8. In **Table 1** it is demonstrated that the Norgen RNA/DNA/Protein Purification Kit consistently isolates RNA with a high 260/230 ratio (typically above 2.0), similar to the leading market competitor. The RNA samples purified using Norgen's kit can therefore be used confidently in any downstream applications, including for highly stringent microarray assays.

The SDS-PAGE analysis indicated that the column-purified proteins isolated using Norgen's kit were of a good diversity and a high quality (**Figure 3**). Protein concentrations were also determined using the Bradford assay (**Table 2**). The highest amount of protein observed was for samples purified using Norgen's sample preparation kit. More importantly, the proteins were column-purified without the use of any alcohol precipitations. Thus, Norgen's kit offers a rapid method to isolate high quality RNA, DNA and proteins from a single sample with no need for fractionation.

In order to assess the quality of the isolated RNA, RT-qPCR was performed (**Figure 4**). A primer set against the human GAPDH gene was used for the amplification.

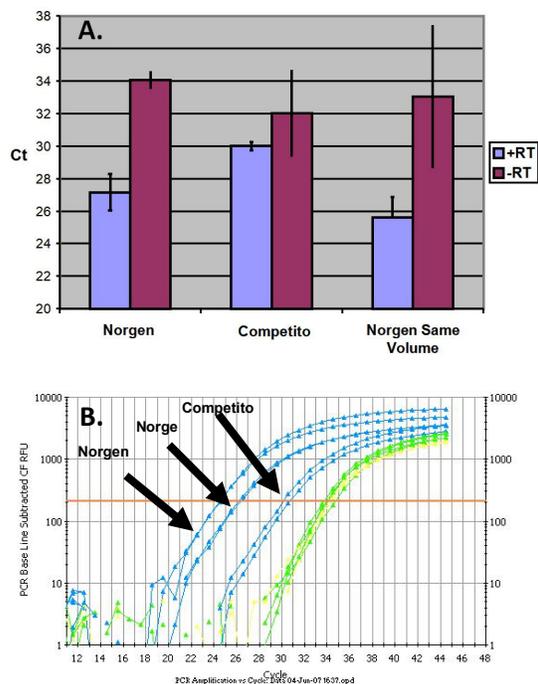


Figure 4. Detection of GAPDH using RT-qPCR from Purified RNA Samples. Total RNA was isolated from HeLa cells using Norgen's purification kit or the leading market competitor's kit and 0.5 µg (5 µL for competitor and 2 µL for Norgen) of the RNA was used as template in an RT-qPCR reaction for the detection of GAPDH. A reaction using the same volume (5 µL) of template RNA as the competitor's was also set up. Panel A shows the Ct values obtained for each of the samples in the presence and absence of reverse transcriptase. GAPDH was detected at a lower Ct value for both input volumes of the Norgen purified RNA versus the competitor's Ct value. Panel B shows the graph of PCR amplification versus cycle for each of the sample types.

RNA isolated from HeLa cells was used as the template in an RT reaction with and without reverse transcriptase in the absence of DNase treatment. The RT reaction was then used in an RT-qPCR reaction to detect the GAPDH gene. **Figure 4** demonstrates that in the RT-qPCR reactions Norgen purified samples (for both input levels) gave much better amplification than did the competitor's RNA sample (lower Ct values in the presence of reverse transcriptase). In addition, all samples had similar Ct values to the no template control in the absence of reverse transcriptase enzyme, indicating that the purified RNA samples had relatively little or no genomic DNA contamination. The total RNA purified using Norgen's RNA/DNA/Protein Purification Kit was of a high purity and had retained its biological activity.

The quality of RNAs isolated from HeLa cells by Norgen's RNA/DNA/Protein Purification Kit was further demonstrated by capillary gel electrophoresis (**Figure 5**). RNA purified from HeLa cells by both Norgen's sample preparation kit and the competitor's kit were resolved on the Agilent RNA Nano 6000 chip. Both samples resolved well on the Lab-on-a-Chip. However, only Norgen's kit allowed for the isolation of small RNA, while the competitor did not isolate any small RNA species (**Figure 5, Panel B**). Furthermore, since there is no sample splitting with Norgen's kit, Norgen resulted in a much higher RNA yield and the purified RNA samples had a better RIN value in comparison to the competitor (**Table 3**).

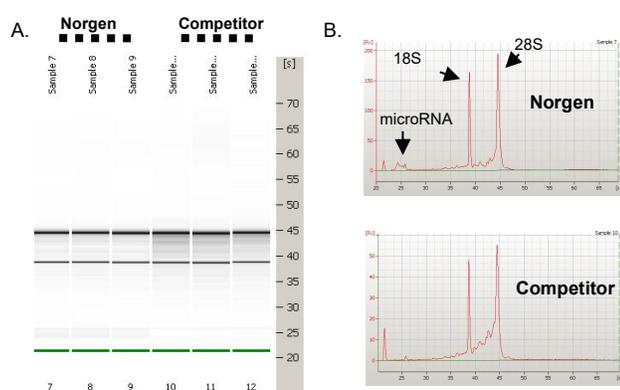


Figure 5. Resolution of Purified RNA Samples on the Agilent BioAnalyzer. Total RNA was isolated from HeLa cells with Norgen's RNA/DNA/Protein Purification Kit and the competitor's kit and resolved on an Agilent Lab-on-a-Chip (Panel A) and electropherograms were generated (Panel B). It shows that the purified RNA is of a high purity and integrity, and that only Norgen's kit resulted in the purification of small RNA species.

Table 3. Quantification of RNA by the Agilent BioAnalyzer.

| Sample Preparation Kit | RNA Concentration (µg/µL) | RNA Yield (µg) | RIN |
|------------------------|---------------------------|----------------|-----|
| Norgen | 0.30 | 14.8 | 9.1 |
| Competitor | 0.11 | 5.3 | 8.9 |

The quality of the proteins purified was evaluated by Western Immunoblotting (**Figure 6**). **Figure 6** showed that strong signals were detected for HeLa total proteins for both sample preparation kits. For both the Norgen kit and the competitor kit the isolated proteins were of high quality and have retained their biological activity.

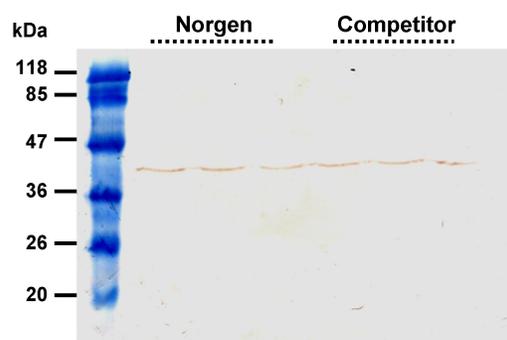


Figure 6. Detection of GAPDH by Western Immunoblotting. Proteins purified using Norgen's kit or the competitor kit were isolated in triplicate and run on a 10% SDS-PAGE gel and then transferred to a PVDF membrane. Western blotting was performed using an anti-GAPDH antibody (Competitor). The first lane contains the molecular weight marker. Approximately 7.5 µL of protein was loaded for each sample. Both Norgen and competitor purified proteins gave a similar intensity of detection.

CONCLUSIONS

Through the analysis of the performance of Norgen Biotek's RNA/DNA/Protein Purification Kit in sequentially isolating RNA, DNA and proteins from HeLa cells, and comparing to a leading competitor kit for multi-analyte purification, a number of conclusions regarding Norgen's kit can be made:

1. Norgen Biotek's kit allows for the sequential isolation of RNA, DNA and proteins in less than 35 minutes from a single sample using the same column. The procedure for the sequential isolation is demonstrated in the scheme in **Figure 1**. The protocol uses a minimal number of steps to isolate the 3 different biomolecules. All RNA, DNA and proteins are isolated and purified from the same sample with no splitting of the lysate, thus increasing yield, while reducing inconsistency and variability. All RNA, DNA and proteins are column-purified using the same column, and without any alcohol precipitation.

2. The RNA, DNA and proteins isolated using Norgen's kit are of the highest purity and integrity. The high purity and integrity of the biomolecules is demonstrated in Figures 2 to 6. Figure 2 demonstrates that the gDNA isolated is of high purity and shows no signs of degradation. Similarly, Figures 2, 4 and 5 indicates that the total RNA purified using the Norgen's kit is of a high purity and shows no signs of degradation. In addition, the purified RNA delivers optimal results in downstream applications, including RT-qPCR analyses, indicating the purified RNA has maintained its biological activity with minimal amount of cross-contamination. The purified proteins are also of good quality (Figures 3 and 6) and are suitable for downstream applications such as Western Immunoblotting.

3. Norgen's kit allows for the isolation of total RNA, including microRNA. Norgen's kit allows for the isolation of the total size diversity of RNA, including the small RNA and microRNA, while the competitors kit did not isolate the small RNA species, as can be seen in Figure , Panel B and Figure 5.

REFERENCES

1. Okamoto I, Morisaki T, Sasaki J, Miyake H, Matsumoto M, Suga M, Ando M, and Saya H. 1998. Molecular Detection of Cancer Cells by Competitive Reverse Transcription-Polymerase Chain Reaction Analysis of Specific CD44 Variant RNAs. *Journal of National Cancer Institute*. **90**: 307-315.

