# Isolation and Detection of RNA from as little as a Single Animal Cell Using Norgen's Total RNA Purification Kit

APPLICATION NOTE #11

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## INTRODUCTION

The isolation of high quality total RNA is an important first step in a number of downstream applications including RT-PCR, Northern blotting, primer extension and expression array assays. Traditional methods of total RNA isolation often involve the use of toxic or harmful chemicals, including phenol and chloroform. Often traces of these organic solvents can be found in the purified RNA, resulting in the need to further purify the RNA once it has been isolated. Furthermore, these methods tend to be rather long and tedious, requiring numerous steps to isolate and purify the RNA. Therefore, simple and rapid methods that allow for the isolation of total RNA, including kits based on spin-column chromatography, are being developed.

Norgen's **Total RNA Purification Kit (Cat. 17200)** allows for the rapid isolation and purification of total RNA from a number of various sources, including cultured animal cells, bacterial cells, blood and small tissue samples. The procedure is based on spin column chromatography, using Norgen's proprietary resin as the separation matrix. RNA can be purified from small amounts of starting material, and does not require the use of harmful chemicals. The purified RNA can be used in a number of downstream applications, including RT-PCR and Northern blotting.

In this application note, Norgen's **Total RNA Purification Kit (Cat. 17200)** is compared to a number of competitor's kit on the basis of RNA purity and yield. Furthermore, RNA isolated with Norgen's kit is used in a downstream RT-PCR reaction, indicating the purity and biological activity of the purified RNA.

# MATERIALS & METHODS

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## Total RNA Purification Kit

(Cat. 17200, 37500, 17250, 17270, 24300, 24350, 24370, 24380, Dx17200, Dx24300, Dx24350, Dx24380) For the rapid purification of total RNA - including microRNA - without the use of phenol.

- Extract high quality & quantity total RNA including miRNA
- No phenol step required; isolate all RNA in one fraction
- Bind & elute all RNA irrespective of size or GC content, without bias
- Very sensitive & linear down to a few cells without the need for carrier RNA
- Isolate from a wide variety of specimens
- A select amount of CE-IVD marked versions available
- Purified RNA is suitable for a variety of downstream applications, including Small RNA Sequencing
- Available in a variety of formats to suit your needs
- Purification is based on spin column chromatography that uses Norgen's resin separation matrix

## Bacterial RNA Isolation

RNA was isolated from 1 x $10^9$ E. coli DH5 α cells using Norgen's **Total RNA Purification Kit (Cat. 17200)** as per the provided protocol. Briefly, the bacteria was pelleted by centrifugation, the media removed, and the pellet resuspended in TE buffer containing lysozyme. Lysis solution was then added to the bacterial suspension, followed by the addition of ethanol. The lysate was then loaded onto a provided column. The column was then washed twice with 400 µL of the Wash Solution. In order to elute the purified bacterial RNA, 50 µL of Elution Buffer was spun through the column two times, in order to obtain 100 µL of purified RNA. Bacterial RNA was also isolated using a leading competitor's kit, as per the supplied instruction.

## Mammalian RNA Isolation

RNA was isolated from varying amounts of 293 HEK cells using Norgen's **Total RNA Purification Kit (Cat. 17200).** Briefly, a confluent plate of 293 HEK cells was trypsinized, and the cells were pelleted and resuspended in a small

volume of media. The cells were then counted, and various dilutions were generated such they contained $10^6$, $10^5$, $10^4$, $10^3$, $10^2$, $10^1$, and $10^0$ cells. RNA was then isolated from the various amounts of cells using the provided protocol. Initially, the cells were pelleted and the media removed. The pellet was then resuspended in lysis solution, and ethanol was added to the solution. The lysate was then applied to a provided column, the column washed twice with Wash Solution, and the purified RNA eluted into 50 µL of Elution Buffer.

### RNA Gel Electrophoresis

The purified RNA was run on 1.5% formaldehyde-agarose gels for visual inspection. Generally, 5 µL of each 100 µL elution was run on the gel.

Denaturing PAGE gels were also run for the RNA samples. Purified RNA was treated with formamide RNA loading buffer, by heating at 80°C for 5 minutes. The samples were then loaded onto a 5% urea-PAGE gel, and electrophoresed at 150V.

### Analysis of RNA using Bioanalyzer

In order to analyze the bacterial RNA samples, 1 µL of each sample was loaded onto an Agilent® RNA 6000 Nano Chip and resolved on an Agilent® 2100 Bioanalyzer.

Total RNA yield and 23S/16S ratios were determined using the supplied Agilent® Bioanalyzer 2100 Biosizing software.

### RT-PCR Assay

An RT-PCR was performed on the RNA isolated from the varying amounts of 293 HEK cells in order to investigate the purity of the RNA, as well as its ability to be used as a template in downstream reactions. The RT-PCR

assay was based on the work of Kreuzer et al (1999). The primers generated allowed for the detection of ß-actin, and the RT-PCR was performed using these primers and the conditions outlined by Kreuzer et al (1999). Ten µL of the final PCR was then run on a 1.5% agarose gel for visual inspection.

## RESULTS & DISCUSSION

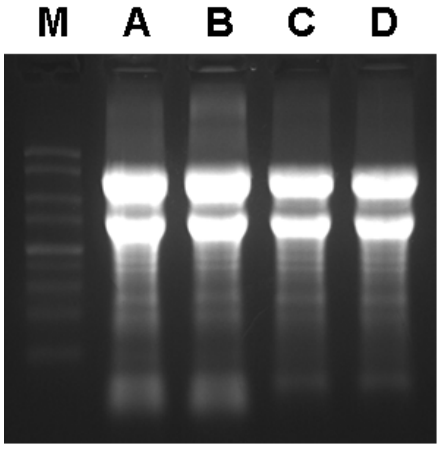


**Figure 1. Formaldehyde-agarose gel of RNA isolated from 1 x $10^9$ E. coli cells. Lane M is an RNA marker, Lanes A and B contain RNA isolated using Norgen's Total RNA Purification Kit and Lanes C and D contain RNA isolated using a competitor's kit.**

In order to evaluate Norgen's RNA Isolation Kit, RNA was initially isolated from 1 x $10^9$ DH5 α bacterial cells using Norgen's kit and a leading competitor's kit. Each protocol was performed in duplicate. Once the RNA was isolated, it was run on a formaldehyde-agarose gel for visual inspection. In each case, 5 µL of the total 100 µL elution was loaded onto the gel. As **Figure 1** indicates, both the 23S and 16S RNA can be seen in both the Norgen-isolated and competitor-isolated RNA, indicating that the kits allow for the isolation of the main RNA species. In both cases the isolated RNA is intact, with no signs of degradation. The main difference that can be detected in **Figure 1** is the presence of small RNA species in the lanes corresponding to RNA isolated using Norgen's kit. The competitor's kit

does not allow for the isolation of as many small RNA species. These small RNA species have recently been found to play a large role in gene regulation, particularly in repression of gene expression. These small RNAs may include short interfering (si) RNAs, small temporal (st) RNAs, heterochromatic siRNAs, tiny noncoding RNAs, and microRNAs (Finnegan and Matzke, 2003). Much attention is currently being given to these small RNAs, and thus it would be desirable when isolating total RNA from a species that these small RNAs are also isolated. Thus, Norgen's kit offers a clear benefit over the competitor's kit in that it is able to isolate greater quantities of these small RNA species.

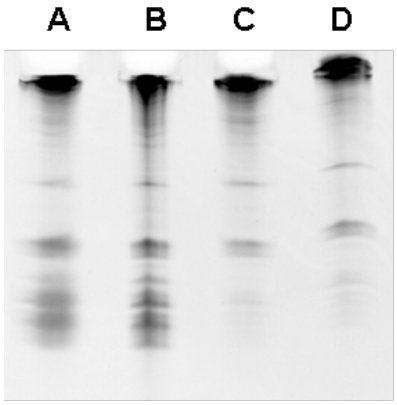


**Figure 2. Denaturing PAGE gel of RNA isolated from 1 x $10^9$ E. coli cells. Lanes A and B contain RNA isolated using Norgen's Total RNA Purification Kit and Lanes C and D contain RNA isolated using a competitor's kit.**

In addition to running the formaldehyde-agarose gel for visual inspection, a denaturing PAGE gel of the isolated RNA was also run. **Figure 2** shows the PAGE gel containing RNA isolated from bacterial cells in duplicate using Norgen's Total RNA Purification Kit and a main competitor's kit. Again the figure shows that the isolated RNA is intact in all cases, with no signs of any degradation. Furthermore, it can again be seen that a greater amount of small RNA species are being isolated using Norgen's kit (Lanes A and B) then using the competitor's kit (Lanes C and D). These small RNA species play an important role in gene regulation, and thus researchers are often interested in isolating and studying these particular RNA species. Again, Norgen's kit allows for the isolation of these important small RNA species, while the competitor's kit does not.

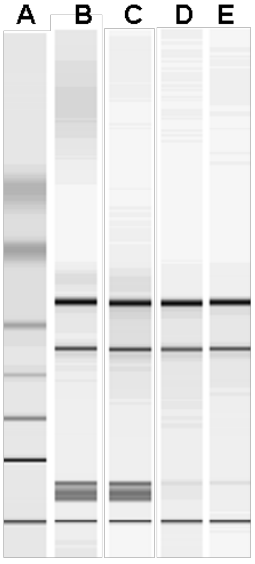


**Figure 3. Resolution of RNA isolated from 1 x 109 E. coli cells using an Agilent® 2100 Bioanalyzer. Lane A corresponds to an RNA marker, Lanes B and C correspond to RNA isolated using Norgen's Total RNA Purification Kit, and Lanes D and E correspond to RNA isolated using a competitor's kit.**

After the isolated RNA was run on the gels, it was also analyzed using the Agilent® 2100 Bioanalyzer. **Figure 3** shows the resolution of the RNA after it was loaded onto an Agilent® RNA 6000 Nano Chip. Again, the gel indicates that the RNA is intact, and that the 2 main RNA species from the bacteria are being isolated.

Furthermore, the presence of small RNAs can again be detected in the lanes containing Norgen-isolated RNA (Lanes B and C). Once the gel was run, further analysis was performed using the Agilent Bioanalyzer 2100 Biosizing software. Using this software, it was determined that the total RNA yield using Norgen's kit was 101.5 µg, while the total yield from the competitor's kit was 60.5 µg. Thus, Norgen's kit allows for the isolation of greater amounts of RNA from the same starting amount of bacterial cells. Software analysis also indicated that the 23S/16S ratio for both the samples was 1.57. This ratio is relatively high, indicating again that the RNA isolated using both kits has a high integrity, and that the RNA in both cases is not degraded.

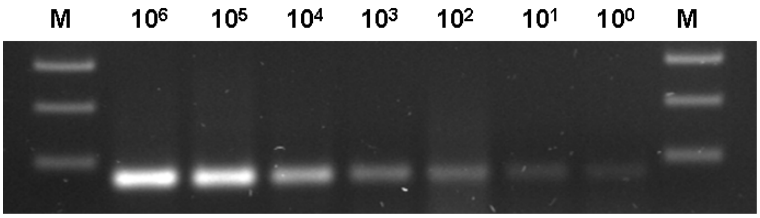


**Figure 4. RT-PCR assay of mammalian RNA isolated from 293 HEK cells using Norgen's Total RNA Purification Kit. M corresponds to a molecular weight marker, while the numbers correspond to the number of 293 HEK cells that were used for the RNA isolation procedure.**

After the competitor comparison was completed, **Norgen's Total RNA Purification Kit (Cat. 17200)** was analyzed to determine if the isolated RNA could be used in downstream applications, as well as the minimum number of mammalian cells from which RNA could be isolated and detected. Mammalian 293 HEK cells were grown, trypsizined, and diluted such that $10^6$, $10^5$, $10^4$, $10^3$, $10^2$, $10^1$, and $10^0$ cells were present in the different dilutions. Norgen's kit procedure was then followed to isolate the RNA from these mammalian cells. Once the RNA was isolated, it was used in an RT-PCR reaction that was based on the work of Kreuzer et al (1999). The RT-PCR was performed, and the resulting 175 bp products were run on a 1.5% agarose gel. In all cases, the RT-PCR was successful, indicating that the purified RNA was intact and of a high integrity. As can be seen in **Figure 4**, it was further found that RNA could be isolated and detected from as little as a single mammalian cell (Lane $10^0$). This result indicates that Norgen's **Total RNA Purification Kit (Cat. 17200)** has a very high sensitivity, and that not only can RNA be isolated from a single cell, but that the RNA is intact and biologically active, as demonstrated by its use as the template in an RT-PCR reaction.

## CONCLUSION

Through the analysis of Norgen's Total RNA Purification Kit, as well as the comparison with a leading RNA isolation kit on the market, a number of conclusions regarding Norgen's kit can be made.

1. **Norgen's kit allows for the isolation of small RNA species.** This can be demonstrated by examining Lanes A and B in Figure 1, Lanes A and B in Figure 2, and Lanes B and C in Figure 3. In all cases, the presence of large amounts of small RNAs can be detected. In contrast, these small RNAs are absent in the lanes corresponding to RNA isolated using the main competitor's kit.

2. **Norgen's kit is extremely sensitive.** This was demonstrated through the isolation and detection of RNA using RT-PCR from as little as a single cell (Figure 4, Lane $10^0$).

3. **Norgen's kit isolates large quantities of RNA.** Norgen's kit allowed for the isolation of 101.5 µg of RNA, while the leading competitor's kit isolated only 60.5 µg of RNA from the same amount of starting material.

Thus Norgen's **Total RNA Purification Kit (Cat. 17200)** is an excellent tool for isolating large quantities of biologically active total RNA from various sources.

## REFERENCES

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| Total RNA Purification Kit Dx | 50 Preps | Dx17200 |
| Total RNA Purification Kit Dx (High Throughput) | 2 x 96-Well Plates | Dx24300 |
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