

Mole[®]

RNA extraction from cultured mammalian cells

“ GeneMole[®] is a benchtop instrument for automated nucleic acid purification. With reduced hands-on time, automation allows users time to focus on other operations as well as reduced exposure to harmful reagents. GeneMole[®] can process 1-8 samples in one run and all the reagents required are available as pre-filled disposable MoleStrips[™]. ”

Introduction

The GeneMole[®] instrument together with the MoleStrips[™] RNA Cells Kit, can be used for automated extraction of total RNA from mammalian cell cultures. The isolation procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. After magnetic separation and removal of the supernatant, the paramagnetic beads are washed several times with different wash buffers to remove contaminants and salts. Finally, highly purified RNA is eluted and is ready for use in downstream applications.

Working with RNA

The condition of the starting material is crucial for the quality of the extracted RNA. Endogenous RNases must be inactivated immediately upon cell death to prevent RNA degradation. To accomplish this, either disrupt and homogenize the sample directly in the presence of RNase-inhibiting or denaturing reagents, or “flash freeze” the sample in liquid nitrogen for later extractions.

Autoclaving tips, tubes and solutions is not sufficient to inactivate RNases. To ensure intact, high quality RNA, it is essential to reduce exposure to environmental RNases.

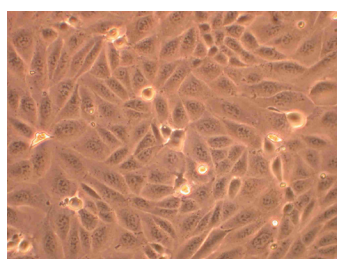


Figure 1
MDCK (Madin Darby canine kidney) cells.

Wear gloves at all time and be sure that all surfaces, including pipettors, benchtops, glassware, and gel equipment are decontaminated with a surface decontamination solution. When working with purified RNA, ensure samples remain on ice during downstream applications.

Methods

Both freshly-prepared cell pellets or cells stored at -80°C are suitable as input material. The GeneMole[®] instrument and MoleStrips[™] RNA Cells Kit have been used for automated isolation of RNA from various mammalian cell lines, some examples are shown below.

Typically, RNA can be extracted from lysates containing between $0.5-1 \times 10^6$ cells. In order to prepare the lysate, mix the cell pellet in $375 \mu\text{l}$ lysis buffer by pipetting. The lysis buffer is provided with the MoleStrips[™] RNA Cells Kit and contains RNase-inhibiting and denaturing reagents. Homogenizing the samples with a “bead beater” (e.g. Precellys 24, Bertin Technologies) give better results than the syringe and needle technique.

Centrifuge the lysate for 1 minute at 10,000 rpm and transfer $350 \mu\text{l}$ of the supernatant to the GeneMole[®] sample tubes. Finally, add $5 \mu\text{l}$ glycogen to each sample and place the tubes in the GeneMole[®] worktray.

Cell Type	HeLa	Hek293	Ramos	MDCK	M1	Sum102	HCT	MCF7	3T3
Input	1x10 ⁶	1x10 ⁶	1x10 ⁶	1x10 ⁶	1x10 ⁶	1x10 ⁶	1x10 ⁶	0.5x10 ⁶	1x10 ⁶
Conc [ng/μl], DNase	40-160	180-210	30-50	80-130	65-75	55-65	200	90-220	30-50
Conc [ng/μl], no DNase	90-230	200-260	60-70	150-170	100-120	80-90	270	200-350	50-70

Table 1

Optimal amount of starting material and the expected RNA yields for various mammalian cell lines when eluting in 100 μl elution buffer. RNA integrity number (RIN) of listed samples were all above 9 for both +/- DNase-treatment protocols.

Results

The optimal input of cells and the final RNA yield will vary according to the size and composition of the cells and also as a result of their biological metabolism. MoleStrips™ RNA Cells has been developed to extract RNA from up to 1 million cells. Table 1 shows the recommended amount of starting material and the yields for different cell lines.

Using intact RNA is a key element for success in downstream applications such as microarray or RT-PCR analysis. The RNA extracted with a GeneMole® instrument, typically has RNA integrity numbers (RIN) above 9, making it suitable for most gene expression studies.

Currently, no available purification method can guarantee that the extracted RNA is completely free of DNA. As PCR can detect even a single molecule of DNA, to avoid false positives and high background, RNA samples should be digested with DNase before RT-PCR reactions. To prevent interference by DNA, we recommend, when possible, designing primers that anneal at intron splice junctions as this will prevent genomic DNA being amplified.

In order to be flexible, two protocols have been optimized for the GeneMole® instrument, with one protocol including automated DNase treatment within the isolation procedure. Figure 2 shows the results of RNA extracted from MDCK cells using both protocols, analyzed by agarose gel electrophoresis.

The success of the DNase treatment was verified in a RT/-RT real-time PCR experiment. Additionally, tests on purity and stability have been performed, showing extracted RNA samples free of contaminants and of high purity and quality.

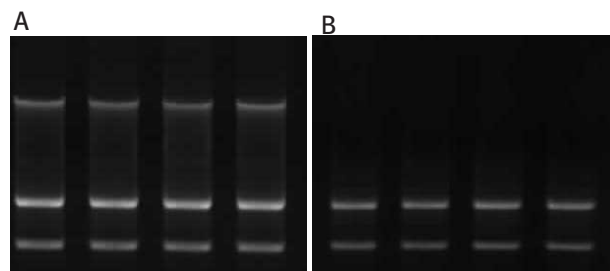


Figure 2

1% agarose gel of (5μl) RNA eluates extracted from MDCK. (A) Results using the protocol without DNase treatment. (B) Results using the protocol including DNase treatment.

Comments

The quality of the starting material must always be considered when evaluating quantity and quality of the extracted RNA.

Do not overload the system. Overloading will significantly reduce yield and purity.

Conclusion

The GeneMole® instrument is an ideal instrument for automated RNA extraction. It strongly reduces hands-on time and provides nucleic acids of high quality.

“Simplify When Possible”

Ordering Information

Product	Content	Product Number
GeneMole®	Instrument	MG10-000-000
MoleStrips™ RNA Cells	64 Reagent Strips	MGK30-102-102
MoleStrips™ RNA Cells Convenience Kit ¹	32 Reagent Strips	MGK30-102-101

¹ The convenience kit contains 32 Reagent Strips and all the required tips, tubes and caps.