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Isolation of total RNA from a human cell line (HEK 293) using TRIzol® in Eppendorf Tubes® 5.0 mL

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Abstract

Since RNA isolation using TRIzol is freely scalable, sample volumes may fall between 2 and 5 mL, depending on the starting material. Such preparations cannot be performed in the classic microcentrifuge tubes up to 2 mL. In this Application Note it will be shown that these volumes may be prepared easily, and with good yield, in the Eppendorf Tubes® 5.0 mL. Their design provides these tubes with a special advantage over 15 mL conical tubes; it allows user friendly handling, combined with contamination-free pipetting.

Introduction

Purification of nucleic acids represents the basis for a broad spectrum of advanced molecular applications. The required amounts of DNA or RNA are obtained from sufficient starting materials. Procedures based on the "single-step" method [1], which have given rise to reagents such as TRIzol, are advantageous as they may be adapted to virtually any amount of starting material and are thus very flexible. However, one limitation is imposed by the available tube formats. In this Application Note, 4 mL of starting material are used for the isolation of RNA using TRIzol; after the addition of further required reagents, the volume will have increased to 4.8 mL. Established standard protocols routinely reach sample volumes which exceed a volume of 2 mL, thus precluding the use of the convenient 1.5/2.0 mL tube format [2, 3]. In these cases, conical 15 mL tubes are often employed. With a diameter of 1.7 cm and a length of approximately 12 cm, they are equipped with a screw cap.
The length of these tubes prescribes that, for the purpose of pipetting, the pipette cone needs to be inserted into the tube in order to reach the bottom. During this process, the pipette cone may easily come into contact with the inside wall of the tube, which carries a high risk of contamination. The conical tubes may be centrifuged at approx. 6,000 – 15,000 x g, depending on the manufacturer and the type; in contrast, the centrifugation stability of microcentrifuge tubes is much higher, typically in the range between 20,000 and 30,000 x g. For these reasons, the centrifugation steps may need to be extended in order to achieve an acceptable result when using conical tubes.
These disadvantages may be avoided with the Eppendorf Tube 5.0 mL. It represents a handy alternative in the volume range between the common 2 mL and 15 mL tubes. Their maximum centrifugation stability is 25,000 x g, which may be advantageous during applications such as precipitation of nucleic acids. Compared to centrifugation at lower g-forces, either the centrifugation time may be shortened, or the yield (recovery rate) will increase [4].
The following experiment describes the isolation of RNA using TRIzol, with the aim of comparing the Eppendorf Tube 5.0 mL to a standard conical 15 mL vessel with regards to RNA yield and handling.

Materials and methods

RNA isolation

The isolation of RNA was performed in duplicate in Eppendorf Tubes 5.0 mL and in conical 15 mL tubes, respectively, in accordance with the protocol provided by the manufacturer of TRIzol [5]. To this end, the adherent cell line HEK 293 was seeded in cell culture flasks and cultured for 3 days. For lysis, 1 mL of TRIzol was added for every 10 mm^2 of culture surface. The lysates of several flasks were pooled in order to create equal conditions for each type of tube. The transfer of 4 mL of the starting material into each tube was followed by the addition into each tube of 800 µL of chloroform, according to the TRIzol protocol. For precipitation of RNA from the aqueous phase 2 mL of isopropanol and for the following wash step 4 mL of ethanol (75 %) were used. Deviating from the protocol,
the RNA precipitation and wash steps were carried out at 20,913 x g in the 5.0 mL tubes. The resulting RNA pellet was then resuspended in 50 µL of DEPC-treated water.

Analysis of the RNA

The yield of the isolated RNA was determined using the Eppendorf BioPhotometer® plus in conjunction with Eppendorf UVettes, and averages were calculated. Furthermore, the RNA samples obtained from the same tube type were pooled, diluted 1:4, and separated on a 1 % denaturing agarose gel in order to visualize the quality and sizes of the individual RNA subunits.

Results and discussion

Table 1 lists the average photometric values of the RNA samples. Taken together, a yield of 10.8 µg/µL of RNA was obtained from the Eppendorf Tube, and 9.0 µg/µL of RNA were obtained from the conical 15 mL tube. These results are equivalent to 540 µg and 450 µg of total RNA, respectively, in an elution volume of 50 µL.

1 µL or 2 µL of the isolated RNA were loaded onto the denaturing 1 % agarose gel (fig. 1). All lanes clearly show the bands of the 28 S and 18 S subunits of the ribosomal RNA.

Table 1: Concentrations of the isolated RNA samples

Tube type	RNA concentration [µg/µL]
Eppendorf Tube 5.0 mL	10.8
Conical 15 mL tube	9.0

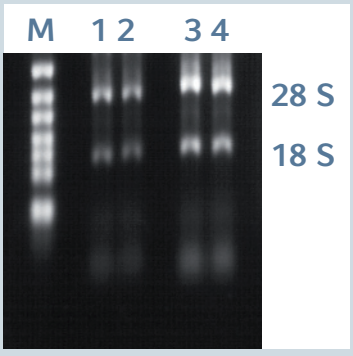

Figure 1: RNA on a denaturing 1% agarose gel
M: RNA Ladder
1: 1 µL RNA from Eppendorf Tube 5.0 mL
2: 1 µL RNA from 15 mL conical tube
3: 2 µL RNA from Eppendorf Tube 5.0 mL
4: 2 µL RNA from 15 mL conical tube

The slightly higher yield achieved with the 5 mL tube compared to the 15 mL tube could possibly be attributed to the increased centrifugation speed which could be applied to both precipitation and wash steps.
The Eppendorf Tube 5.0 mL showed clear advantages with respect to handling. In contrast to the conical 15 mL tube, it was possible with the Eppendorf tube to perform all pipetting steps without inserting the pipette cone into the tube. Thus, there was no risk of inadvertent transfer of sample material by the pipette. Furthermore, opening and closing of the snap lid was significantly more convenient than the cumbersome handling of the screw cap of the 15 ml conical tube. The shape of the lid of the 5 mL tube, when aligned in the centrifuge in a defined manner, was helpful during the subsequent location of translucent pellets.

Conclusion

The Eppendorf Tube 5.0 mL could be used successfully for the isolation of RNA. In comparison with the conical 15 mL tubes, a yield which was at least comparable was achieved. Importantly, significant advantages were observed for handling, as all steps could be carried out comfortably in the 5.0 mL Tube while avoiding contamination.

References

[1] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162(1):156-159.
[2] Sambrook J, Fritsch EF and Maniatis T. Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Laboratory Press; 1989.
[3] Ausubel F, Brent R, Kingston R, Moore D, Smith JA, Seidman J, and Struhl K. Current protocols in molecular biology. New York: Greene Publishing Associates; 1996.
[4] Eppendorf Application Note 234: Centrifugation at 30,000 x g in plasmid DNA precipitation allows better recovery rates and shorter centrifugation times (http//:www.eppendorf.com).
[5] User manual TRIzol® Reagent (www.invitrogen.com).

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Tubes® 5.0 mL, Eppendorf Quality, 200 tubes	0030 119.401	0030119401
Eppendorf Tubes® 5.0 mL, PCR clean, 200 tubes	0030 119.460	0030119460
Eppendorf Tubes® 5.0 mL, Sterile, 200 tubes	0030 119.487	0030119487
Eppendorf Tubes® 5.0 mL, Biopur®, 50 tubes (individually wrapped)	0030 119.479	0030119479
Eppendorf Protein LoBind Tubes 5.0 mL, PCR clean, 100 tubes	0030 108.302	0030108302
Eppendorf DNA LoBind Tubes 5.0 mL, PCR clean, 200 tubes	0030 108.310	0030108310
Tube Clip 5.0 mL, 10 pcs., secures lid for boiling	0030 119.509	0030119509
Starter Pack Eppendorf Tubes® 5.0 mL, PCR clean, 400 tubes, 2 racks (16 spaces), white, 8 universal adapters for rotors with bore for 15 mL conical tubes	0030 119.380	0030119380
Eppendorf BioPhotometer® plus	6132 000.008	952000006
UVette®, Original Eppendorf UV-transparent plastic cuvette, individually wrapped, PCR clean, certified, 80 pcs.	0030 106.300	952010051
UVette® routine pack, Eppendorf Quality purity, re-sealable box, 200 pcs.	0030 106.318	952010069

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