In vitro Transcribed RNA Cleanup with Mag-Bind® TotalPure NGS

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Introduction

The application of RNA in therapeutics, diagnostics and analytical methods has significantly increased in recent years. Improvements in manufacturing throughput and turnaround time are required for design and synthesis of RNA molecules to realize their immense clinical potential. To answer this critical need, Tebubio laboratories offer an RNA production service providing µg amounts of custom RNA in as little as 5 days, to be used for screening purposes in preclinical research (vaccine, therapeutic agent, personalized medicine, etc), pharmaceutical industry, biotechnology companies, and academia.

As experts in the field, Tebubio laboratories have tested the use of Omega Bio-tek's Mag-Bind® TotalPure NGS Kit (M1378) to cleanup RNA synthesized following an in vitro transcription. The current application note demonstrates the ability of Mag-Bind® TotalPure NGS Kit (M1378) to efficiently purify RNA and reports the RNA yield at different bead-to-sample ratios.

Materials and Methods

In vitro transcription reactions of 20 µL volume were cleaned up with Mag-Bind® TotalPure NGS beads (M1378) using a bead-to-sample volume ratio of 0.5X, 1.0X, 1.5X, 2.0X, 2.5X, and 3.0X. After binding for 15 minutes at room temperature (RT), beads were washed twice with 200 µL of 70% ethanol, air dried for 5 minutes at RT and finally eluted with 60 µL RNase-free water following incubation for 15 minutes at RT.

For comparison, an equivalent in vitro transcription reaction of 20 µL volume was purified using a silica-based column with a binding capacity of 200 µg according to the manufacturer's instructions.

The quality and quantity of RNA recovered were verified by NanoVue™ Plus (GE Healthcare) and TapeStation® 4150 (Agilent).

Results and Discussion

The demonstrated protocol is capable of processing up to 96 samples in ~45 minutes. Concentration, quantity, and absorbance ratios of the eluted RNA are shown in Table 1. The results indicate that a bead-to-sample volume ratio of 2.0X resulted in the best yield, higher than the silica-based column.

The TapeStation® profile of the 2.0X bead-to-sample volume cleanup ratio verifies the high quality and integrity of the eluted RNA (Figure 1).

Table 1. RNA recovery post cleanup using Mag-Bind® TotalPure NGS Kit at different bead-to-sample volume ratios as compared to cleanup using silica spin columns. RNA yield was calculated based on the total recovered eluate volume of 50 µL.

	Mag-Bind® TotalPure NGS beads						
	0.5X	1.0X	1.5X	2.0X	2.5X	3.0X	Silica Spin Column
RNA Concentration (ng/µL)	1522	2276	3426	3928	3916	3763	3062
A_{260}/A_{280}	2.1	2.1	2.0	1.9	1.9	1.9	2.1
A_{260}/A_{230}	2.3	2.2	2.1	1.9	1.9	2.0	2.1
RNA Yield (µg)	76	114	171	196	196	188	153

TapeStation® Analysis of RNA Post Cleanup

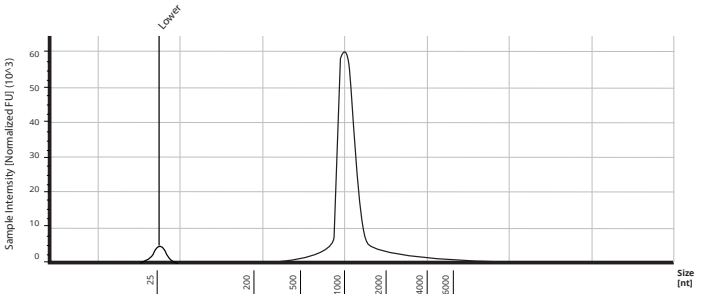

Figure 1. Quality assessment of purified RNA from the Mag-Bind® TotalPure NGS Kit was performed using a TapeStation® 4150 system from Agilent.

Conclusions

Based on RNA recovery and quality, Tebubio laboratories validated the performance of Mag-Bind® TotalPure NGS beads (M1378) for RNA cleanup. The demonstrated protocol is user-friendly, rapid (approximately 45 minutes for up to 96 samples), and tunable. The entire protocol can be automated on most open-ended liquid handling platforms allowing high throughput cleanup of RNA, thus providing significant benefit in RNA manufacturing.

Product Information

Product No.	Description
M1378-00	Mag-Bind® TotalPure NGS beads (5 mL)
M1378-01	Mag-Bind® TotalPure NGS beads (50 mL)
M1378-02	Mag-Bind® TotalPure NGS beads (500 mL)

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