Application Note

RiboShield® RNase Inhibitor protects RNA during *in vitro* transcription reactions

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Introduction

In vitro transcription (IVT) is a widely used technique for generating RNA molecules for research, diagnostics, and therapeutic development. One of the key challenges during RNA production is the ubiquitous risk of RNase contamination, which can rapidly degrade RNA products. PCR Biosystems' RiboShield® RNase Inhibitor provides robust protection against RNase A, RNase B, and RNase C contamination, ensuring maximum RNA integrity during workflows.

A critical consideration when using RNase inhibitors is whether their use interferes with enzymatic processes such as transcription. This application note evaluates whether RiboShield® has any effect on IVT yield when added to transcription reactions using a commercial kit.

Materials & Methods

Reaction Setup

In vitro transcription was performed using the MEGAscript Kit (Thermo, Cat. no. AM1330). Reactions were prepared in duplicate under the following conditions:

- Control (no RiboShield®)
- +10 U RiboShield® per reaction
- +40 U RiboShield® per reaction

Each 20 µL reaction contained:

1. 10x Reaction Buffer (2 µL)
2. NTP mix (8 µL; 2 µL each of ATP, CTP, GTP, UTP)
3. RNA Polymerase Enzyme Mix (2 µL)
4. Linearised plasmid template (1 µL, positive control from kit)
5. RiboShield® (0, 0.25, or 1 µL of 40 U/µL stock)
6. Nuclease-free water to volume

Reactions were incubated at 37 ˚C for 2 h or 18 h.

For the RNase protection assay, 1 µg RNA samples were supplemented with 2 U/µL RiboShield® RNase Inhibitor in 20 µL reactions and incubated with increasing amounts of RNase A (0-160 pg) at 37 ˚C for 30 min and analysed by agarose gel.

Analysis

Agarose gel electrophoresis: 1 µL of each transcript was diluted with 9 µL MQ water. 10 µL NEB RNA loading buffer (Cat. no. B0363A) were added to the sample and tubes were incubated at 70 ˚C for 15 min before loading them on a 1% Agarose Gel (in TEA buffer). Ladder 1:4 (Thermo, AM7150) was also used. The gel was run at 70 V for about 2 h. RNase protection assays were also run on a 1% agarose gel in a similar manner.

Spectrophotometry ($OD_{260/280}$): Transcripts were diluted 1:100 in Nuclease-Free Water (Cat. no. PB40.41). RNA concentration was estimated by measuring the OD at 260 and 280 nm. To calculate the concentration in µg/µL the following formula was used: OD_{260} x 10 (mm) x 100 (dilution factor) x 0.04 µg/µL, because a standard solution of RNA at 40 ng/ µL has an OD_{260}= 1. The OD_{260} to OD_{280} ratio was used to assess sample purity, and should be ≥1.8.

Results

RNA yields were comparable across control and RiboShield®-supplemented reactions. Both 2 h and 18 h IVT reactions showed no reduction in RNA yield when RiboShield® was present at 10 U or 40 U per reaction (Table 1).

Sample	OD_{260}	Concentration µg/µL	$OD_{260/280}$
Control 2 h	0.214	8.56	1.87
+10 U 2 h	0.211	8.43	1.80
+40 U 2 h	0.207	8.26	1.83
Control 18 h	0.203	8.14	1.95
+10 U 18 h	0.213	8.53	1.94
+40 U 18 h	0.198	7.92	1.95

Table 1: *In vitro* transcription yields ± RiboShield®RNase Inhibitor

Agarose gel analysis confirmed strong and consistent RNA bands, with no evidence of inhibition or degradation of the resulting RNA (Figure 1).

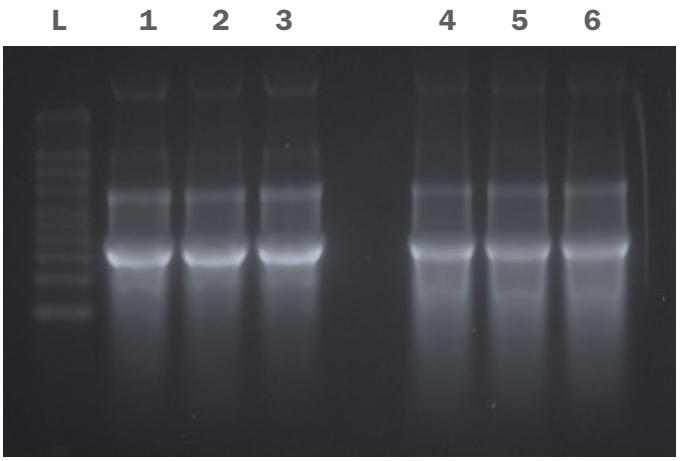

Figure 1: Transcribed RNA resulting from IVT reactions with and without RiboShield® RNase Inhibitor.

L: ladder, 1: Control 2 h, 2: +10 U RiboShield® 2 h IVT, 3: +40 U RiboShield® 2 h IVT, 4: Control 18 h, 5: +10 U RiboShield® 18 h IVT, 6: +40 U RiboShield® 18 h IVT.

In order to test the effectiveness of RiboShield® in protecting RNA, 1 µg RNA samples were supplemented with RiboShield® and incubated with increasing amounts of RNase A (0-160 pg per sample) for 30 min at 37 ˚C. Samples were than analysed by agarose gel electrophoresis (Figure 2). RiboShield® prevented any obvious degradation up to 10 pg RNase A and reduced degradation considerably up to 40 pg RNase A.

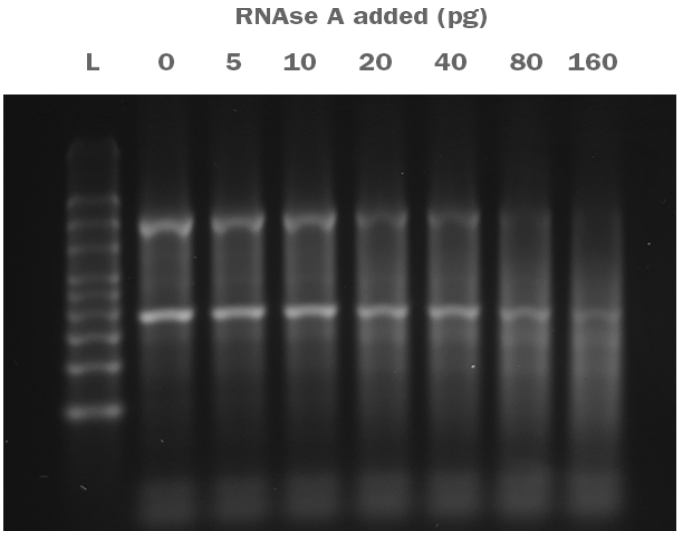

Figure 2: 2 U/µL RiboShield® RNase Inhibitor provides superior protection against up to 40 pg RNase A.

RiboShield® RNase Inhibitor was incubated with the indicated amounts of RNase A and 1 µg RNA at 37˚C for 30 min and analysed on an agarose gel. L: Ambion RNA Millennium Marker.

Conclusion

These results demonstrate that RiboShield® RNase Inhibitor can be confidently added to *in vitro* transcription reactions to protect RNA from RNase degradation without compromising transcription efficiency.

Recommendations for use

We recommend adding 40 units of RiboShield® RNase inhibitor to a 20 µL IVT reaction (1 µL per reaction to achieve a final concentration of 2 U/µL).

Ordering Information

Please reach out to our team with any queries or to get a quote for RiboShield® RNase Inhibitor by email: info@pcrbio.com. Please refer to the Table 2 below for available pack sizes and catalogue numbers.

Reactions	Presentation (reactions)	Cat. No.
RiboShield® RNase Inhibitor	2500 units	PB30.23-02
	10000 units	PB30.23-10

Table 2: Pack sizes and catalogue numbers of RiboShield® RNase Inhibitor