

DNase I Recombinant, RNase-free High-Quality Tool for Demanding Applications

Cordula Nemetz* and Claudia Vorwerk

Roche Applied Science, Penzberg, Germany

*Corresponding author: cordula.nemetz@roche.com

Introduction

Preparation of RNA, free from DNA, is a critical step before performing RT-PCR assays. Total RNA isolated from several sources using routine methodologies is frequently contaminated with DNA that can give rise to amplification products, which mimic the amplicons expected from RNA targets. DNase I recombinant, RNase-free safely and efficiently eliminates genomic DNA contaminations from RNA samples.

Complete Digestion of Contaminating DNA

DNase I recombinant, RNase-free has a specific volume activity of 10×10^3 U/ml. To quantify the digestion efficiency of the new recombinant DNase I, a real-time PCR experiment with primers specific for β -globin and dsDNA-specific SYBR green dye was performed using the LightCycler® Instrument.

For this purpose, the β -globin gene was amplified from 100 ng of human genomic DNA (both from the LightCycler® Control Kit DNA), either with or without previous digestion with 1 unit of DNase I recombinant, RNase-free. The crossing point values of both reactions were analyzed and compared with the values obtained from standard dilution series (data not shown).

Figure 1 shows a shift of the crossing point from 20.15 without DNase I to a value of 40.64 after DNase I treatment, corresponding to less than 1 pg of genomic DNA. This intense shift of the real-time PCR signal shows the high efficiency and superior elimination of DNA contamination at the detection limit.

Digestion of Genomic DNA Previous to RT-PCR

For the detection of RNA transcripts by RT-PCR, it is a prerequisite to remove contaminating genomic DNA from the RNA samples. In the following RT-PCR experiments, DNase I, RNase-free was used to digest residual DNA after preparation of RNA from HepG2 human hepatoma cells. For this purpose, incubation of the RNA fraction with DNase I was followed by a heat-inactivation step.

Figure 2 shows the agarose gel analysis of the reactions after RT-PCR with primers specific for human β -actin. A specific 587 bp single-band product was observed after digestion of the RNA with both native and recombinant DNase I, RNase-free. Without DNase digestion, an unspecific by-product probably resulting from genomic contamination was detected.

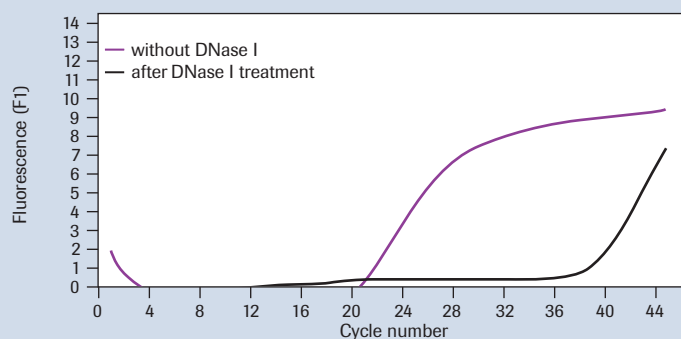


Figure 1: Amplification of β -globin from genomic DNA with and without treatment by DNase I, RNase-free.

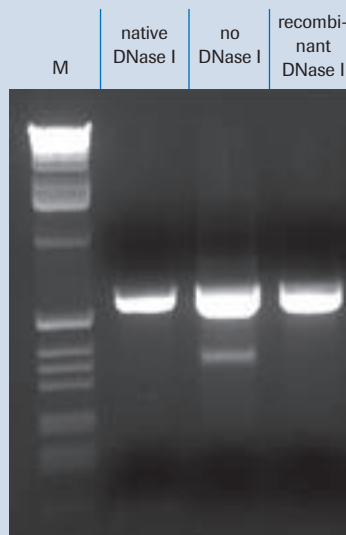


Figure 2: RT-PCR experiment using primers specific for β -actin.

RNA was prepared from HepG2 cells using the High Pure RNA Isolation Kit without the on-column digestion step. 20 μ l of the eluate were incubated with 4 U DNase I, RNase-free (native enzyme), DNase I recombinant, RNase-free or without DNase I. 5 μ l of the RNA solution were subjected to reverse transcription using Transcriptor Reverse Transcriptase and random hexamer primers in a volume of 20 μ l. The β -actin gene was amplified from 5 μ l of the obtained cDNA using a block cycler.

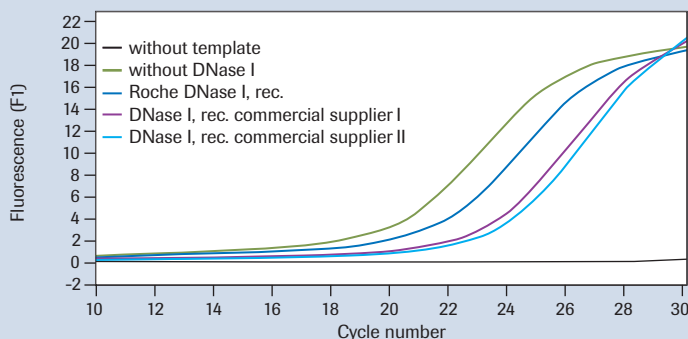


Figure 3: Real-time RT-PCR experiment using the LightCycler® h-ALAS Housekeeping Gene Set. RNA was prepared from HepG2 cells using High Pure RNA Isolation Kit without the on-column digestion step. 20 µl of the eluate remained either untreated, or were incubated with 4 U of DNase I recombinant, RNase-free, or recombinant DNase I of commercial suppliers I or II for 10 minutes at 37°C and heat inactivated at 75°C for 10 minutes. 5 µl of the RNA solution were subjected to reverse transcription using Transcriptor Reverse Transcriptase and random hexamer primers in a volume of 20 µl. Subsequently, a real-time PCR was performed using a h-ALAS specific primers and HybProbe probes.

In another experiment, the performance of DNase I recombinant, RNase-free, was tested for real-time RT-PCR. After digestion of the RNA with DNase I as described above, reverse transcription and real-time amplification were performed using the LightCycler® h-ALAS Housekeeping Gene Set. As shown in Figure 3, RNA samples digested with DNase I recombinant, RNase-free, gave early positive PCR signals, demonstrating the integrity of the RNA.

A Recombinant Enzyme You Can Trust

Rely on a recombinant animal-free enzyme with minimized level of contaminants. Trust the lot-to-lot consistency ensuring reproducible results.

This highly purified enzyme meets and even exceeds the performance of native bovine material, by exhibiting lower RNase and protease activities, and being free of DNA.

It is available in a convenient solution at 10 U/µl. A specially formulated 10x incubation buffer is provided with the enzyme. ■



Product	Pack Size	Cat. No.
DNase I recombinant, RNase-free	10,000 U (10 U/µl)	04 716 728 001
LightCycler® h-ALAS Housekeeping Gene Set	1 set (96 reactions)	03 302 504 001
LightCycler® 2.0 Instrument	1 instrument	03 531 414 201
LightCycler® Control Kit DNA	1 kit (50 reactions)	12 158 833 001
Transcriptor Reverse Transcriptase	250 U (25 reactions)	03 531 317 001
	500 U (50 reactions)	03 531 295 001
	2,000 U (200 reactions)	03 531 287 001
High Pure RNA Isolation Kit	1 kit (50 reactions)	11 828 665 001

Imprint



Roche Diagnostics GmbH
Roche Applied Science
Sandhofer Straße 116
D - 68305 Mannheim

Managing Editor: Dr. Burkhard Ziebolz
Editorial Team: Dr. Friedhelm Hübner
Dr. Ulrich Jellinghaus
Padma Rao

Biochemica is published quarterly by Roche Applied Science and Springer Medizin Verlag and is intended to disseminate information that is of interest and useful to the research community.

We always welcome your comments and suggestions. Contact your local Roche representative for a FREE subscription or to change your address.

Biochemica 2, 2006, Cat. No. 04 800 222 001



Springer Medizin Verlag GmbH
Corporate Publishing
PD Dr. Beate Fruhstorfer,
Ulrike Hafner, Ursula Hilpert,
Dr. Friederike Holthausen,
Sabine Jost, Dr. Claudia Krekeler,
Dr. Christine Leist, Sabine Lohrengel (resp.)
Katrin Stobbe

We welcome manuscripts on any life-science topic that involves Roche Applied Science products used in novel techniques, improvements of common techniques, or simplified protocols. Manuscripts and correspondence concerning editorial matters should be addressed to:

Springer Medizin Verlag
Editorial Office Biochemica
Sabine Lohrengel
Tiergartenstraße 17
D - 69121 Heidelberg
E-mail: sabine.lohrengel@springer.com

© Springer Medizin Verlag, Heidelberg 2006

ISSN 0946-1310