DNase I Treatment Kit

(version A1)

Catalog No. 25502

Active Motif North America
1914 Palomar Oaks Way, Suite 150
Carlsbad, California 92008, USA
Toll free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351

Active Motif Europe
Avenue Reine Astrid, 92
B-1310 La Hulpe, Belgium
UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050

Active Motif Japan
Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733

Active Motif China
787 Kangqiao Road
Building 10, Suite 202, Pudong District
Shanghai, 201315, China
Telephone: (86)-21-20926090
Hotline: 400-018-8123

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Overview

The DNase I Treatment Kit offers an efficient method to remove contaminating genomic DNA from purified RNA samples. RNA samples are treated with DNase I, an endonuclease that non-specifically cleaves single-stranded and double-stranded DNA and the DNA strand of RNA:DNA hybrids.

When performing RNA analysis methods such as reverse-transcription PCR (RT-qPCR) or RNA sequencing, it is important to eliminate all traces of genomic DNA that could have an effect on downstream analysis. RT-qPCR is a highly sensitive method that is commonly used for gene expression analysis. The mRNA template is reverse transcribed into cDNA and then PCR amplified. Any genomic DNA that was carried over during the RNA purification process could serve as a template for PCR amplification and reduce the accuracy of the expression analysis. Similarly, the presence of DNA during RNA-seq analysis will reduce the number of desired RNA sequencing reads.

The DNase I Treatment Kit includes enough reagents for the DNase treatment and purification of 30 RNA reactions. For upstream RNA purification, we recommend the use of Active Motif's RNA Subcellular Isolation Kit.

product	format	catalog no.
DNase I Treatment Kit	30 rxns	25502
RNA Subcellular Isolation Kit	30 rxns	25501

Kit Performance and Benefits

The DNase I Treatment Kit is designed to eliminate DNA from RNA samples.

Maximum Column Binding Capacity	40 µg
Maximum Column Loading Volume	750 µl
Size of purified RNA	RNA between the sizes of 75 nt to 7,000 nt

DNase I Treatment Kit

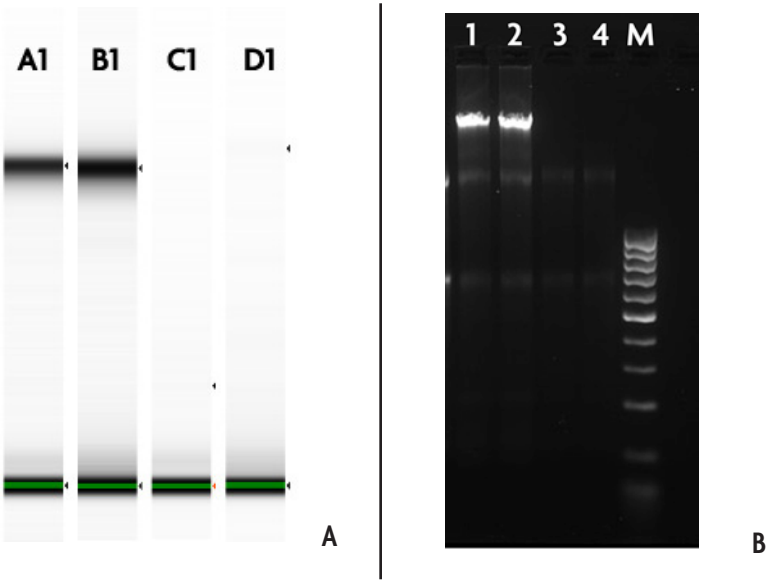

Figure 1: Comparison of Nuclear RNA sample before and after DNase I treatment.

Image A shows an Agilent TapeStation DNA tape with 1 µg nuclear RNA samples before DNase I treatment (Lanes A1 & B1) and following DNase I treatment (Lanes C1 & D1). Image B shows 1 µg of the same nuclear RNA samples before DNase I treatment (Lanes 1-2) and following DNase treatment (Lanes 3-4) with a 100 bp molecular weight marker. Both images demonstrate the efficiency of the DNase I treatment at removing genomic DNA from the RNA sample.

DNase I Treatment Kit Components and Storage

DNase I Treatment Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
DNase I	60 µl	-20°C
DNase I Stop Solution	60 µl	-20°C
10X DNase I Reaction Buffer 2	60 µl	-20°C
Buffer G	20 ml	4°C
Purification Columns	30 ea	4°C
DEPC Water	10 ml	RT

Additional materials required

- PCR tubes and thermocycler
- 70% Ethanol
- β-mercaptoethanol
- RNase-free microcentrifuge tubes and microcentrifuge
- Vortex set to highest setting
- Ice bucket and ice
- Nanodrop, Qubit or similar device for RNA quantification
- RNA sample

Buffer Preparation and Recommendations

Preparation of Complete Buffer G

Buffer G contains salts that will precipitate out of solution during cold temperature storage. We recommend placing the buffer at room temperature 1 hour prior to use to allow the solution to warm up. Ensure that the salts are fully dissolved into solution before use by vortexing at room temperature. Prepare the amount of Complete Buffer G required for the assay. Transfer the necessary amount of Buffer G to a clean container. Add 10 µl of β-mercaptoethanol per 1 ml Buffer G (see the Quick Chart for Preparing Buffers in this section). Mix well by vortexing. Keep the buffer at room temperature during the assay. After use, discard remaining Complete Buffer G.

Preparation of Wash Buffer

Prepare the amount of Wash Buffer required for the assay. Transfer the necessary amount of Buffer G (without β-mercaptoethanol) for the wash steps into a clean container. Add an equal volume of 70% ethanol (*e.g.* Add 5 ml 70% ethanol to 5 ml Bufer G). Invert repeatedly. The buffer can be stored at room temperature after the addition of ethanol.

Quick Chart for Preparing Buffers

Reagents to prepare	Components	For 1 rxn	For 10 rxns	For 30 rxns
Complete Buffer G	β-mercaptoethanol	3 µl	33 µl	100 µl
	Buffer G	297 µl	3.27 ml	9.9 ml
	TOTAL REQUIRED	**300 µl**	**3.3 ml**	**10 ml**
Wash Buffer	70% Ethanol	300 µl	3.3 ml	10 ml
	Buffer G	300 µl	3.3 ml	10 ml
	TOTAL REQUIRED	**600 µl**	**6.6 ml**	**20 ml**
70% Ethanol	**TOTAL REQUIRED**	**1.25 ml**	**13 ml**	**40 ml**
DEPC Water	**TOTAL REQUIRED**	**50 µl**	**500 µl**	**1.5 ml**

Assay Protocol

Read the entire protocol before use.

Section A: DNase I Treatment

The protocol below describes the treatment of 2 µg RNA per reaction. Scale the protocol accordingly if working with different quantities of RNA such that 1 µl DNase I is used per 1 µg RNA sample and 1 µl DNase I Stop Solution is used per 1 µg RNA. It is important not to vortex the reactions when the DNase I is added as this can greatly reduce the efficiency of the DNase I.

1. To a PCR tube, add the following reagents in the order listed below:

Component	20 µl Rxn
10X DNase I Reaction Buffer 2	2 µl
DNase I	2 µl
RNA sample	2 µg
DEPC Water	Up to 20 µl
TOTAL	**20 µl**

2. Mix samples up and down by pipetting. DO NOT VORTEX. Quick spin to collect contents to the bottom of the tube.
3. Incubate at room temperature for 15 minutes.
4. Add 2 µl DNase I Stop Solution to each tube. Pipette up and down to mix.
5. Incubate at 65°C for 10 minutes in a thermocycler.
6. Remove samples from the thermocycler and place on ice.

Section B: RNA Purification

1. Transfer each reaction to a microcentrifuge tube. Label each tube.
2. Add 300 µl Complete Buffer G to each sample.
3. Vortex tubes on highest setting for 30 seconds.
4. Add 350 µl of 70% ethanol to each tube.
5. Using a pipette and P200 tip, pipette up and down to homogenize the solution.
6. Prepare separate purification columns for each sample. Add the entire sample to its corresponding purification column.
7. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.

8. Add 600 µl Wash Buffer to each column.
9. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
10. Add 600 µl of 70% ethanol to each column.
11. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
12. Repeat centrifugation at 14,000 rpm for 2 minute at 4°C to remove any remaining liquid.
13. Transfer each column to a new RNase-free microcentrifuge tube. Make sure the new collection tubes are labeled for each sample.
14. Add 50 µl DEPC water to the center of each column. Let stand for 1 minute.
15. Centrifuge the columns at 14,000 rpm for 1 minute at 4°C. Eluate contains the purified RNA.
16. Discard the columns. Place RNA samples on ice.
17. Proceed to Section C for RNA quantification.

Section C: RNA Quantification

1. Quantify RNA using the absorbance 260nm value with a Nanodrop or equivalent spectrophotometric device. If anticipating low RNA yield, use a fluorescent RNA detection method, such as Qubit, to quantify the RNA samples. For accurate quantification it is important that the sample measurement falls within the detectable range of the instrument used.
2. We recommend preparing smaller aliquots of RNA and storing the samples at -20°C to -80°C. It is best to avoid repeated freeze/thaw cycles when working with RNA.

Section D: Troubleshooting Guide

Problem/question	Recommendation
Little or no recovery of RNA	Make sure to use RNase-free tubes, tips and reagents when working with RNA samples. Wear gloves at all times when handling the samples.
	Quantify RNA using a spectrophotometer or fluorescent detection method. For accurate quantification it is important that the sample measurement falls within the detectable range of the instrument used.
I still see DNA in my sample	It is important not to vortex the DNase I reaction. This will greatly reduce the efficiency of the DNase I treatment. The protocol provided is for the treatment of 2 µg of RNA per reaction. If working with larger amounts, scale up the reaction quantities accordingly.
Is it normal for my sample consistency to change after addition of 70% ethanol?	Yes, the combination of the Complete Buffer G and the 70% ethanol will change the consistency of the sample. Use a P200 pipette and tip and mix the sample by pipetting up and down 15-20 times. The sample consistency should become easier to work with after pipetting.
Storage of RNA	RNA samples may be stored at -20°C to -80°C. We recommend preparing smaller aliquots of RNA to avoid freeze/thaw cycles..

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

Active Motif North America

1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008, USA
E-mail: tech_service@activemotif.com

Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351

Active Motif Europe

Avenue Reine Astrid, 92
B-1310 La Hulpe, Belgium
E-mail: eurotech@activemotif.com
Direct: +32 (0)2 653 0001

Germany Free Phone: 0800 181 99 10
France Free Phone: 0800 90 99 79
UK Free Phone: 0800 169 31 47
Fax: +32 (0)2 653 0050

Active Motif Japan

Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan

Direct: +81 3 5225 3638
Fax: +81 3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China

787 Kangqiao Road
Building 10, Suite 202
Pudong District
Shanghai, 201315, China

Direct: (86)-21-20926090
Hotline: 400-018-8123
E-mail: techchina@activemotif.com

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