

SunScript™

Reverse Transcriptase
RNaseH-



ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
SunScript™ Reverse Transcriptase RNaseH-	10 reactions	421010
SunScript™ Reverse Transcriptase RNaseH-	50 reactions	422050
SunScript™ Reverse Transcriptase RNaseH-	200 reactions	423200

KIT CONTENTS

DESCRIPTION	10 REACTIONS	50 REACTIONS	200 REACTIONS
SunScript™ RT RNaseH- (70 U/μl)	15 μl	75 μl	300 μl
5X Reaction Buffer	40 μl	200 μl	800 μl
0.1 M DTT	20 μl	100 μl	400 μl

SOURCE

E.coli production strain harbouring expression constructs for SunScript™.

ACTIVITY UNIT DEFINITION

One unit incorporates 1 nmol of dTMP into a Poly(A)-oligo(dT)₁₂₋₁₈ template in 10 min at 37°C.

INACTIVATION

Inactivated by incubating 95°C for 10 min.

SHIPPING AND STORAGE

This product is shipped in dry ice. Upon receipt, it should be stored immediately at -20°C in a non-frost-free (constant temperature) freezer. If stored correctly, the product can be kept for at least six months after shipping without displaying any reduction in performance. For longer periods of time, store the kit at -80°C.

HANDLING

Always wear gloves when working with RNA to avoid contaminations from human skin. Change them frequently, especially after touching skin, surfaces, etc. Use RNase free materials and reagents. Glassware should be heat-treated (250°C O/N). In doubt, rinse containers with 0.1 N NaOH/1 mM EDTA and then DEPC-treated water. Solutions should be treated by adding DEPC to 0.05%, incubating overnight and autoclaving. Design an area in the laboratory where to work exclusively with RNA, and use a separate set of pipets only for RNA work. For more specific information please consult the Material Safety Data Sheets (MSDS) available online at www.expedeon.com.

QUALITY CONTROL ASSAYS

Absence of endonuclease and exonuclease

SunScript™ RT RNaseH- has been determined to be free of detectable endonucleases, exonucleases and nicking activity. A fluorogenic substrate designed to react with all these kind of nucleases has been incubated in the presence of 1 µg enzyme for 1 hour at 37°C. No fluorescence increase above the negative control was detected.

Absence of ribonucleases

SunScript™ RT RNaseH- has been determined to be free of detectable single-strand ribonuclease activity. A fluorogenic substrate designed to react with these kind of nucleases has been incubated in the presence of 1 µg enzyme for 30 min at 37°C. No fluorescence increase above the negative control was detected.

Purity

The purity of the enzyme has been determined to be higher than 95% by SDS-polyacrylamide gel electrophoresis and densitometric measurements.

Functional assay

SunScript™ RT RNaseH- and the reagents of this kit have been tested in an RT-PCR assay for successful reverse transcription of a 16 Kb mRNA target using oligo-dT primers.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY THE USER

- Sterile nuclease-free tubes, pipettes and pipette tips.
- Microcentrifuge
- Thermocycler
- Vortexer
- dNTPs
- Optional: (single-stranded) RNase inhibitors
- RNase-free water

FIRST STRAND cDNA SYNTHESIS PROTOCOL

The following protocol is optimized to synthesize first strand cDNA to use in subsequent PCR.

1. Thaw the reaction components, mix and briefly centrifuge. Keep on ice.
2. Add the following components into a sterile, nuclease-free tube:

total RNA	10 pg - 1 µg	
or		
poly(A)+ RNA	10 pg - 500 ng	1 µl
Oligo(dT) ₁₈₋₂₀	10 -100 pmol	
or		
Random primers	10 - 100 pmol	
or		
Specific primers	10 - 20 pmol	1 µl

3. Incubate the mixture at 68°C for 5 min. Put on ice.
4. Collect the contents of the tube by centrifugation and add the following components:

5X Reaction Buffer	4 µl
0.1 M DTT	2 µl
10 mM each dNTP	1 µl
40 U RNase Inhibitor (optional)	X µl
RNase-free water	X µl
SunScript™ RT RNaseH-	1.5 µl
	Final volume = 20 µl

5. Mix gently and incubate at 65°C for 30-60 min. (If especially complex secondary structures of the RNA are suspected, or for complete representation of different RNA species, the incubation temperature can be increased up to 85°C. It is recommended to extend the temperature to 70 and 75°C first as there will be some activity drop beyond 75°C).
6. Stop the reaction by incubating at 95°C for 10 min.

The resulting cDNA can be used directly for subsequent applications, or stored at -20°C or -80°C. Avoid multiple freeze-thaw cycles.



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