



4BB™ Lyophilized SunScript®

Reverse Transcriptase

HANDBOOK



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ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
4BB™ Lyophilized SunScript® Reverse Transcriptase	8 reactions	424008
4BB™ Lyophilized SunScript® Reverse Transcriptase	48 reactions	425048
4BB™ Lyophilized SunScript® Reverse Transcriptase	200 reactions	426200

KIT CONTENTS

DESCRIPTION	8 REACTIONS	48 REACTIONS	200 REACTIONS
4BB™ Lyophilized SunScript® Reverse Transcriptase	1 x 8-Strip PCR Tube	6 x 8-Strip PCR Tube	25 x 8-Strip PCR Tube
5X Reaction Buffer	50 µl	220 µl	4 x 220 µl

SOURCE

E.coli production strain harbouring expression constructs for 4BB™ 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 at 95°C for 10 min.

SHIPPING AND STORAGE

This product is shipped at room temperature. 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. Once opened for use, 5X Reaction Buffer tubes should be stored separately at -20°C. Lyophilized strips can be stored at -20°C inside the same zip bag that contained them, with caution to leave the silica gel inside the bag.

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 overnight). 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.4basebio.com.

QUALITY CONTROL ASSAYS

Absence of Endonuclease and Exonuclease

4BB™ lyophilized SunScript® Reverse Transcriptase 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 30 min at 37°C. No fluorescence increase above the negative control was detected.

Absence of Ribonucleases

4BB™ Lyophilized SunScript® Reverse Transcriptase 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.

Absence of Proteases

4BB™ Lyophilized SunScript® Reverse Transcriptase has been determined to be free of detectable protease activity. A fluorogenic substrate designed to react with these kind of proteases has been incubated in the presence of 1 µg enzyme for 1h 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

4BB™ Lyophilized SunScript® Reverse Transcriptase 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. For each 4BB™ Lyophilized SunScript® Reverse Transcriptase 8-Strip PCR Tube, prepare the following mixture:

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

3. Incubate the mixture at 68°C for 5 min. Put on ice
4. Take a 4BB™ Lyophilized SunScript® Reverse Transcriptase 8-Strip PCR Tube, collect the contents of the tubes by centrifugation and prepare the following mix:

5X Reaction Buffer	36 µl
10 mM each dNTP	9 µl
40 U RNase Inhibitor (optional)	X µl
RNase free water	X µl
Final volume = 162 µl	

Split 18 µl of this MIX in each tube of the strip.

5. Add 2 µl of the mixture stored on ice of Step 3 into each tube of the strip. 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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