



4BB™ SunScript®  
Reverse Transcriptase  
RNaseH-  
HANDBOOK



## INDEX

Ordering Information .....	3
Kit Contents .....	3
Source .....	3
Activity Unit Definition .....	3
Inactivation .....	3
Shipping and Storage .....	3
Handling .....	3
Quality Control Assays .....	4
Reagents and Equipment to be Supplied by the User .....	4
First Strand cDNA Synthesis Protocol .....	4

Use and Use Restrictions: The Products are sold, and deliverables of any services are provided for the purposes of the buyer's internal *in vitro* research, development or educational use only, not for *in vivo*, or any therapeutic or diagnostic use, nor for resale, or for providing services or any other commercial use of any kind, including without limitation, for any transfer in any form (including as part of a kit) to a third party; for analysis or reverse engineering of the Product or for manufacturing. Products should only be used in accordance with any safety data sheets, guidance or protocols that we issue from time to time and are available for download from our Site. Protective clothing should be used at all times when handling our Products. Safety datasheets relating to all Products are available for download from the Site or upon request. 4basebio grants no other license or rights under any intellectual property in respect of Products or services deliverables and in particular grants no license to use any Product or deliverables for any commercial purposes. Sale of Products or service deliverables by us or our authorised distributors are expressly conditional upon the customer's agreement with these restrictions, which the customer gives upon placing an order for Products or deliverables. If you wish to use any Product or deliverables for any purpose other than your own internal research as described above, you will require an additional licence from 4basebio. Please contact [info@4basebio.com](mailto:info@4basebio.com).

## ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
4BB™ SunScript® Reverse Transcriptase RNaseH-	10 reactions	421010
4BB™ SunScript® Reverse Transcriptase RNaseH-	50 reactions	422050
4BB™ SunScript® Reverse Transcriptase RNaseH-	200 reactions	423200

## KIT CONTENTS

DESCRIPTION	10 REACTIONS	50 REACTIONS	200 REACTIONS
4BB™ SunScript® RT RNaseH- (70 U/μl)	20 μl	85 μl	330 μl
5X Reaction Buffer	50 μl	220 μl	4x220 μl
0.1 M DTT	440 μl	440 μl	440 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)<sub>12-18</sub> 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.4basebio.com](http://www.4basebio.com).

## QUALITY CONTROL ASSAYS

### Absence of endonuclease and exonuclease

4BB™ 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 30 min at 37°C. No fluorescence increase above the negative control was detected.

### Absence of ribonucleases

4BB™ 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

4BB™ 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 - 1µg	
or		
poly(A)+ RNA	10 pg - 500 ng	1 µl
Oligo(dT) <sub>18-20</sub>	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
4BB™ 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.



**4basebio**

**4basebio**

[orders@4basebio.com](mailto:orders@4basebio.com)

[www.4basebio.com](http://www.4basebio.com)