# SunScript®

## One Step RT-qPCR Kit

HANDBOOK

4basebio

# INDEX

## ORDERING INFORMATION

| PRODUCT | SIZE | CAT. NO. |
|---|---|---|
| SunScript® One Step RT-qPCR Kit | 100 reactions | 450100 |

## KIT CONTENTS

| DESCRIPTION | CAP COLOR | VOLUME |
|---|---|---|
| Enzyme Mix | Purple | 1 x 45 µl |
| 2x Reaction Buffer | Yellow | 1 x 1000 µl |
| $H_2O$ | Blue | 1 x 1000 µl |

## 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 three months after shipping without displaying any reduction in performance. For longer periods of time, store the kit at -80°C. Avoid longer exposure of kit contents to light. Avoid frequent freeze-thaw cycles.

## 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 pipettes only for RNA work

All chemicals should be considered as potentially hazardous. This material may contain substances or activities that are harmful to human health. It should not be ingested, inhaled, or brought into contact with skin, and handled with appropriate care in accordance with the principles of good laboratory practice. In case of contact with skin wash immediately with water. For more specific information please consult the Material Safety Data Sheets (MSDS) available online at www.4basebio.com.

## QUALITY CONTROL

Each batch of SunScript® One Step RT-qPCR Kit and individual components are tested against predetermined specifications to ensure consistent product quality.

## REAGENTS AND EQUIPMENT TO BE SUPPLIED BY THE USER

- Sterile nuclease-free tubes, pipettes and pipette tips.
- Microcentrifuge
- Real time PCR instrument
- Vortexer

## DESCRIPTION

SunScript® One Step RT-qPCR Kit is an easy and reliable system designed for fast RT-qPCR (real time) reactions based on detection of double-stranded DNA-binding dyes. One main advantage of the kit is the elevated temperature for performing the RT step thus allowing higher specificity of the reaction, and a higher likelihood in obtaining reaction products for difficult targets. Moreover, the excellent kinetic performance of the SunScript® Reverse Transcriptase allows for speed optimization of the protocol.

The kit contains all the components needed to perform both reverse transcription and qPCR amplification in the same reaction by using gene specific primers, in a "one step" reaction. This minimizes contaminations and allows higher reproducibility.

The system uses reaction mixes containing SunScript® Reverse Transcriptase RNaseH+, a high quality HotStart Taq Polymerase, RNase inhibitor, and SYBR Green I, a double-stranded DNA-binding dye for a robust and effective quantification of RNA molecules. Intercalated SYBR Green I fluorophore can be detected using common settings for SYBR Green I (excitation wavelength ~450 nm, emission wavelength~ 520 nm).

SunScript® Reverse Transcriptase RNaseH+ is an engineered version of the well characterized HIV-1 RT with increased thermostability. It minimizes RNA degradation for higher cDNA yields and allows to perform the reverse transcription step at temperatures up to 75ºC if needed (the recommended temperature for the RT reaction is 60ºC for most samples and depending on the melting temperature of the primers). These features make this kit the best choice for amplifying more difficult RNAs with a high degree of secondary structure or high GC content.

The reverse transcription reaction can be effectively achieved, while Taq Polymerase remains inactive during this step due to blocking antibodies. The inactivation/activation step at 95ºC provides an automatic "hot start" for the Taq Polymerase to amplify the reverse transcribed cDNA.

The reaction mixes contain proprietary formulations which have been optimized for both the reverse transcription and the qPCR reaction, including dNTPs, MgCl2 and stabilizers (to provide a final concentration of 250 nM each dNTP and 3.5 mM MgCl2 in the reaction).

## PROTOCOL

IMPORTANT: The complete reaction setup must be performed ON ICE or on a cooling plate.

1. Thaw the kit components, and briefly centrifuge. Keep on ice.

2. For each sample, add the following components into a reaction compartment (96-well plate, or strips) compatible with your qPCR instrument (check the manufacturer's recommendations) and keep on ice. Avoid longer exposure to light:

| COMPONENT | VOLUME / REACTION |
|---|---|
| Template RNA | X µl |
| 2x reaction buffer (yellow) | 10 µl |
| Enzyme mix (purple) | 0.4 µl |
| Sense primer (10 µM) | 1 µl |
| Anti-sense primer (10 µM) | 1 µl |
| $H_2O$ (blue) | to a final volume of 20 µl |

For multiple samples it is convenient to pipet first the template RNA in each reaction tube, and then prepare a Master Mix by adding appropriate multiples of the rest of the components. Pipet then the corresponding amount of Master Mix to each reaction tube.

3. Gently mix and make sure that all the components are at the bottom of the tube. Centrifuge briefly if needed. Keep on ice.

4. Real time thermal cycling parameters:

   Remark: If a particularly difficult RNA target is expected, the RNA may be denatured prior to addition to the reaction mix (e.g. heat RNA sample to 90°C for 5 min and put on ice immediately before adding to the other reaction components).

| CYCLING STEP | TEMPERATURE | STANDARD | FAST |
|---|---|---|---|
| 1. RT reaction | 60ºC | 15 min | 10 min |
| 2. Taq activation | 95ºC | 2 min | 2 min |
| 3. PCR (30-40x) | 95ºC | 10 sec | 10 sec |
| 4. Annealing | 60ºC | 30 sec | 30 sec |
| 5. Extension | 72ºC | 30 sec | - |
| 6. Measuring point | 83ºC | 5 sec | 5 sec |
| 7. Melting curve | 65-95ºC | X min | X min |

Table 1. Real time thermal cycling parameters: Suggested standard and fast protocols.

Remarks:

- The optional measuring point is of course dependent on the melting temperature of your target amplicon, 83°C is a good starting point for an amplicon of 200-350 bp.
- Although 60°C is the recommended temperature for the RT reaction under most circumstances, this step can be adjusted up to 75°C if necessary. A temperature gradient protocol can be ideally used to determine the optimal temperature for both the RT as well as the PCR annealing step.

Run your qPCR instrument using settings for SYBR Green I or FAM detection (intercalated SYBR Green I can be detected with an excitation wavelength ~450 nm, emission wavelength~ 520 nm).

# TROUBLESHOOTING GUIDE

| PROBLEM | SOLUTION |
|---|---|
| Reduced yield or no amplification product | **The RNA is damaged or degraded**<br>Check RNA integrity by agarose gel electrophoresis, or capillary gel electrophoresis (Agilent Bioanalyzer etc.). Prepare fresh RNA template taking care to prevent RNase activity.<br><br>**Insuficient amount of RNA template**<br>Increase the amount of template RNA.<br><br>**Primer design is not optimal**<br>Redesign the primers with a primer design software and test them in a control reaction, or use validated primers. Use only gene specific primers. Do not use random primers or Oligo dT primers.<br><br>**Primer concentration is not optimal or primers are degraded**<br>Use primer concentrations between 400 nM-1 µM. Avoid using old dilutions of primers and repeated freeze/thaw cycles.<br><br>**Reverse transcription temperature is not optimal**<br>For most reactions 60°C is suitable for the reverse transcription step, but if this does not work well for your template test temperatures in a range of 50°-70°C in small increments. Use a temperature gradient if your instrument has the capability.<br><br>**Annealing temperature too high**<br>Decrease annealing temperature in 2°C increments. Determine the TM of your primers with a program.<br><br>**Missing reagent or error in protocol setup**<br>Check concentrations, storage conditions, volumes and dilutions of all the reagents. Perform the reaction again and include a positive control.<br><br>**Template RNA has a high degree of secondary structure or GC content**<br>Test temperatures for the RT step from 50°C to 75°C in small increments. |
| Non specific amplification products | **DNA contamination in the sample**<br>Perform a control not including the reverse transcription step. Design primers that anneal in exons flanking an intron or at the exon-exon boundary of the mRNA.<br><br>**Reactions set up at room temperature**<br>Always set up RT-qPCR reactions on ice to prevent degradation of RNA and/or undefined start of the reaction.<br><br>**Primer dimer formation**<br>Avoid complementary sequences at the 3′ end of the primers. Use design software for the oligos.<br><br>**Primers are not specific enough for the target**<br>The primer sequence is not specific to the target, or several cDNA products can be obtained from alternatively spliced genes. Check this and redesign the primers accordingly.<br><br>**Annealing temperature is too low**<br>Increase annealing temperature in 2°C increments. |


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