

# SuPrimeScript qRT-PCR Kit

## (Real-time PCR for TaqMan Probe)

Product Name	Cat. No.	Size
SuPrimeScript qRT-PCR Kit	Q-5000	100 rxn* X 1
SuPrimeScript qRT-PCR Kit (with ROX dye)	Q-5100	100 rxn* X 1

\* For 100 X 20 $\mu$ l reaction

### Package information

Q-5000	10X Enzyme Solution (200 $\mu$ l X 1) - with SuPrimeScript RTase, HS Prime Taq DNA Polymerase and RNase Inhibitor
	2X qRT-PCR Buffer (1.2 ml X 1) - with reaction buffer and dNTPs mixture
Q-5100	10X Enzyme Solution (200 $\mu$ l X 1) - with SuPrimeScript RTase, HS Prime Taq DNA Polymerase and RNase Inhibitor
	2X qRT-PCR Buffer (1.2 ml X 1) - with reaction buffer and dNTPs mixture
	50X ROX dye (25 $\mu$ M, 50 $\mu$ l X 1)

### Description

SuPrimeScript qRT-PCR Kit (for Probe Real-time PCR) provides a complete system for fast, high-yield and reliable single-tube one-step qRT-PCR.

### Usage Information

- The reaction temperature for cDNA synthesis is **50°C**.
- The reaction time for cDNA synthesis is **20 min**.

- **Research Use Only**
- **Store at -20°C**

### Protocol

The following 20 $\mu$ l or 50 $\mu$ l reaction volume can be used for one-step qRT-PCR.

1. Program the real-time PCR instrument.

2. Prepare the reaction mixture

Components		Volume	
RNase-free water		add up to 20 $\mu$ l	add up to 50 $\mu$ l
Upstream Primer (10 pmoles/ $\mu$ l, 10 $\mu$ M)		x $\mu$ l	x $\mu$ l
Downstream Primer (10 pmoles/ $\mu$ l, 10 $\mu$ M)		x $\mu$ l	x $\mu$ l
TaqMan probe (10 pmoles/ $\mu$ l, 10 $\mu$ M)		x $\mu$ l	x $\mu$ l
[50X ROX dye (Option)]*		[x $\mu$ l]	[x $\mu$ l]
RNA	- total RNA (1 ng ~ 500 ng) - mRNA (0.1 ng ~ 50 ng)	x $\mu$ l	x $\mu$ l
2X qRT-PCR Buffer		10 $\mu$ l	25 $\mu$ l
10X Enzyme Solution		2 $\mu$ l	5 $\mu$ l

### ♣ 50X ROX dye

ROX dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is supplied at a 25  $\mu$ M concentration. Use the following table to determine the amount of ROX to use with a particular instrument (per 50 $\mu$ l reaction volume).

Instrument	Amount of ROX per 50 $\mu$ l reaction	Final ROX Concentration
AB 7000, 7300, 7700, 7900HT, 7900HT Fast, StepOne, and StepOnePlus	1.0 $\mu$ l (1X)	500 nM
AB 7500, QuantStudio Stratagene Mx3000P, Mx3005P, and Mx4000	0.1 $\mu$ l* (0.1X)	50 nM

★ To accurately pipet 0.1 $\mu$ l per reaction, we recommend diluting ROX 1:10 immediately before use and use 1 $\mu$ l of the dilution.

3. PCR cycling

Step	Temp. & Time		Cycles
	Temp.	Time	
cDNA synthesis	<b>50°C</b>	<b>20 min</b>	1
Initial denaturation	<b>95°C</b>	<b>10 sec</b>	1
Amplification	95°C 60°C	5 sec 30~45 sec	30 ~ 45