HyperScript™ III Reverse Transcriptase

Introduction

HyperScript™ III Reverse Transcriptase is a third-generation reverse transcriptase based on M-MLV Reverse Transcriptase that has been genetically engineered. HyperScript™ III Reverse Transcriptase generates a reduced RNase H activity and improves thermal stability and fidelity compared to wild-type MMLV. Therefore, it has characteristics of higher cDNA synthesis yield and longer length, higher reverse transcription efficiency for high-GC content RNA.

This product can tolerate higher reaction temperatures and is suitable for reverse transcription of RNA templates with complex secondary structures. In addition, the product has an enhanced affinity for templates, making it suitable for reverse transcription of small amounts of template as well as low-copy genes, and can reverse transcribe cDNA up to 12.3 kb for subsequent PCR, qPCR experiments or other experiments.

Components and storage

Components	2,000 U	10,000 U	40,000 U
HyperScript™ III Reverse Transcriptase (200 U/μL)	10 μL	50 μL	200 μL
5x HyperScript™ III First-Strand Buffer	40 μL	200 μL	800 μL
Store the components at -20℃ for 2 years.			

Protocol

1. **First-strand cDNA Synthesis**

   1.1 RNA denaturation (this step is optional, RNA denaturation helps to open the secondary structure and can greatly increase the yield of synthesized cDNA. If your target length exceeds 3 kb, this denaturation step is essential), prepare the following mixture in an RNase-free PCR tube.

Components	Volume
50 μM oligo(dT)$_{20}$, or 200–500 ng/μL of oligo(dT)$_{12-18}$ or 50-250 ng/μL Random Primers or 2 μM gene-specific primer (GSP)	1 μL
10 pg - 5 μg total RNA or 10 pg-500 ng of mRNA	X μL
10 mM dNTP Mixture	1 μL
RNase free ddH2O	Up to 14 μL
Note: The catalog number of 10 mM dNTP Mixture is K1041.	

Incubate at 65°C for 5 minutes, and quickly place on ice for 1 minute.

1.2 After cooling on ice, collect the contents of the tube by brief centrifugation, then prepare the reverse transcription reaction system:

Components	Volume
mixture from step 1.1	14 μL
5x HyperScript™ III First-Strand Buffer	4 μL
RNase Inhibitor, Murine (40 U/μL)	1 μL
HyperScript™ III Reverse Transcriptase (200 U/μL)	1 μL
Note 1: The catalog number of RNase Inhibitor, Murine is K1046. *Note 2: If your target length is ≥ 5 kd, you can use gene-specific primers, oligo(dT)$_{20}$, or oligo(dT)$_{23}$ V/N, and you can also increase the addition volume of HyperScript™ III Reverse Transcriptase up to 2 μL.*	

1.3 Gently Mix and incubate at 25°C for 5 min if taking Random Primers.

1.4 Incubate at 50°C for 30-60 min. For difficult templates or templates with more secondary structures, you can also increase the reaction temperature to 55 °C especially if you can't generate ideal results at 50°C.

1.5 Inactivate the reaction by heating at 70°C for 15 minutes.

1.6 The products can be used immediately for subsequent PCR or qPCR reactions.

Or you can store at -20°C for a short time, for longer storage, please store at -80°C and avoid repeating freeze-thaw cycles.

However, if you need PCR to amplify some long fragments of interest (>1 kb), you may need to remove RNA complementary to the cDNA. You can add 2 units of E. coli RNase H (K1093, 0.4 μL)

and incubate at 37°C for 20 minutes to remove RNA.

2. **PCR**

The following are the steps to perform a PCR reaction with Taq DNA polymerase using the first-strand cDNA as a template, generally, less than 10% of the first-strand cDNA synthesis reaction volume can be used in PCR, and addition of more templates will not increase the amplification yield and may result in a decrease of the PCR product.

2.1 Prepare a 50 µL reaction (Taq DNA Polymerase Cat. No. K1035) using the table below:

Components	Volume
10X PCR Buffer (Mg^{2+} plus)	5 µL
10 mM dNTP Mixture	1 µL
Forward primer (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
Taq DNA polymerase (5 U/µL)	0.5 µL
cDNA from first-strand reaction	2 µL
ddH_2O	Up to 50 µL

2.2 Gently mix and centrifuge briefly, the reaction procedure is as follows:

Temperature	Time	Cycles
94°C	3 min	1
94°C	30 s	15-40
Tm-5°C	30 s	
72°C	1 min/kb	
72°C	5 min	1
4°C	+∞	1

Notes

1. Storage buffer of HyperScript™ III Reverse Transcriptase: 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) IGEPAL CA630, 50% (v/v) glycerol.
2. If your subsequent experiment is qPCR, you may need the following products:

Catalog number	Product name

K1070	HotStart™ 2X SYBR Green qPCR Master Mix
K1170	HotStart™ Universal 2X SYBR Green qPCR Master Mix
K1171	HotStart™ 2X FAST SYBR Green qPCR Master Mix
K1172	HotStart™ Universal 2X FAST SYBR Green qPCR Master Mix
K1541	HotStart™ 2X Probe qPCR Master Mix
K1542	HotStart™ Universal 2X Probe qPCR Master Mix

3. This product is for scientific research purposes only.

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