HyperScript™ III First-Strand cDNA Synthesis Kit

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. Therefore, it has characteristics of higher cDNA synthesis yield and longer length, higher reverse transcription efficiency for high-GC content RNA.

This kit is based on HyperScript™ III Reverse Transcriptase, contains all the components required for the synthesis of first-strand cDNA, and provides primers for cDNA synthesis: Random Primers and Oligo(dT)$_{23}$VN. Random Primers, Oligo(dT)$_{23}$VN or gene-specific primers can be employed as reverse transcription primers according to your needs, and the synthesized first-strand cDNA products can be used for subsequent PCR amplification, qPCR reactions or other experiments.

Composition and storage conditions

Components	20 rxns (20 μL reaction)	50 rxns (20 μL reaction)	100 rxns (20 μL reaction)
HyperScript™ III Reverse Transcriptase (200 U/μL)	20 μL	50 μL	100 μL
5x HyperScript™ III First-Strand Buffer	80 μL	200 μL	400 μL
RNase Inhibitor, Murine (40 U/μL)	20 μL	50 μL	100 μL
10 mM dNTP Mixture	20 μL	50 μL	100 μL
RNase Free ddH2O	1 mL	1 mL	2 X 1 mL
Random Primers (50 μM)	20 μL	50 μL	100 μL
Oligo (dT)$_{23}$VN (50 uM)	20 μL	50 μL	100 μL
Store the components at -20°C for 2 years.			

Protocol

First-strand cDNA Synthesis

1. RNA denaturation, prepare the following mixture in RNase-free PCR tubes.

Components	Volume
Oligo $(dT)_{23}VN$ (50 μM) or Random Primers (50 μM) or Gene Specific Primers (2 μM)	1 μL
Total RNA (1 pg to 5 μg) or Poly(A) + RNA (1 pg to 500 ng)	X μL
10 mM dNTP Mixture	1 μL
RNase free ddH2O	Up to 14 μL
Note: RNA denaturation is an optional step that helps to open secondary structures and improve reverse transcription efficiency and should not be omitted for targets longer than 3 kb.	

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

2. Collect the contents of the tube by brief centrifugation, then prepare the reverse transcription reaction system.

Components	Volume
mixture from step 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

3. Gently mix, then centrifuge briefly, and set up the reverse transcription program as the following table:

Temperature	Time
25°C*a	10 min
50°C*b	50 min
85°C	5 min
Note: **a. This step is to be set up only when you are taking Random Primers. For Oligo(dT)$_{23}$VN or Gene Specific Primers, this step is not necessary.* ****b**. HyperScript™ III Reverse Transcriptase has a good amplification capability for RNA templates with complex secondary structures, generally, reactions at 50°C are recommended.* *If you can't obtain desired results at 50°C because of difficult templates or templates with a lot of secondary structures, you can increase the incubation temperature to 55°C.*	

4. The reverse transcription 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.

[Note 1] If you need PCR to amplify some longer fragments of interest (>1 kb), you may need to remove RNA that is 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 min.

[Note 2] If the synthesized first-strand cDNA is used as a template for the PCR reaction, the amount of template added will affect the amplification efficiency of the PCR, and the addition amount should be not exceeding 1/10 of the total volume of the PCR reaction.

Primer design

1. If the RNA is derived from eukaryotes, it is recommended to choose Oligo(dT)$_{23}$VN, which pairs with the 3' Poly(A) tail of eukaryotic mRNA for the highest yield of full-length cDNA. For prokaryotic templates, we recommend Random Primers or Gene Specific Primers.
2. If the following experiment is qPCR, a mix of Oligo(dT)$_{23}$VN and Random Primers at a ratio of 1:1 can be used to improve the authenticity and reproducibility of qPCR results.
3. Random Primers have the lowest specificity and highest general applicability, and templates such as mRNA, rRNA, tRNA, and LncRNA can all be reverse transcribed with Random Primers. In general, the recommended number of Random Primers used is 1-2 μL for cDNA shorter than 2 kb, and 0.4-1 μL for cDNA longer than 2 kb.
4. When the templates have complex secondary structures or high GC contents, and the usage of Oligo (dT)23VN or Gene Specific Primers can't lead to ideal results, Random Primers can be used.
5. Gene Specific Primers are the most specific. However, there are some cases where Gene Specific Primers are not effective, in this situation, reverse transcription can be re-performed with Oligo(dT)$_{23}$VN or Random Primers instead.

Notes

1. The experiment should be operated on ice to avoid RNase contamination during the process.
2. The purity of RNA will affect the yield of cDNA synthesis, and attention should be paid to prevent RNA degradation during RNA extraction.
3. 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

4. This product is for scientific research purposes only.

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