HyperScript™ IV First-Strand cDNA Synthesis SuperMix (with gDNA wiper)

Description

HyperScript™ IV Reverse Transcriptase is a genetically engineered fourth-generation reverse transcriptase based on M-MLV and provides superior robustness and reliability in RT reactions. The enzyme has significant improvements in inhibitor resistance, processivity, and reaction speed while retaining all the advantages of HyperScript™ III Reverse Transcriptase, including thermostability, highly efficient full-length cDNA synthesis, and reduced RNase H activity. The product can still provide reliable, consistent, and rapid cDNA synthesis in the presence of inhibitors (residues from RNA extraction).

The kit contains all components for the synthesize of first-strand cDNA and provides two kinds of primers for cDNA synthesis as Random Primer and Oligo(dT)$_{23}$VN. The inclusion of the 4× gDNA wiper in the product is intended to remove potential genomic DNA contamination before reverse transcription. Random Primer, Oligo(dT)$_{23}$VN or gene-specific primer can be chosen as reverse transcription primer according to your needs, and the products can be used in subsequent PCR amplification, qPCR reactions and other experiments.

Components and storage

Components	50 rxns (20 µL reaction)	100 rxns (20 µL reaction)
RNase Free ddH2O	1 mL	2×1 mL
5×HyperScript™ IV SuperMix	200 µL	400 µL
4×gDNA wiper mix	190 µL	380 µL
Random Primers (50 µM)	50 µL	100 µL
Oligo (dT)$_{23}$VN (50 µM)	50 µL	100 µL
5×HyperScript™ IV control Mix	20 µL	40 µL
Store the components at -20℃ for 2 years.		

Protocol

First-strand cDNA Synthesis

1. RNA denaturation: Prepare the following mix in RNase-free PCR tubes.

Components	Volume
Rnase free ddH2O	Up to 11.25 µL
Template RNA	Total RNA: 1 pg-2.5 µg

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

2. Removal of Genomic DNA: Prepare the following mixture, mix gently.

Components	Volume
Mixture from step 1	11.25 µL
4×gDNA wiper mix	3.75 µL

Incubate at 37°C for 2 minutes.

3. Centrifuge briefly, then prepare as the following table:

Components	Volume
Mixture from step 2	15 µL
Oligo $(dT)_{23}VN$ (50 µM) or Random Primers (50 µM) or Gene Specific Primers (2 µM)	1 µL
5×HyperScript™ IV SuperMix	4 µL

4. Control Reaction (Optional)

The control reaction is a reverse transcription negative control without reverse transcriptase. If the reverse transcription product will be used in subsequent qPCR experiments, the control reaction can be used to verify the absence of genomic DNA contamination in the RNA sample. Prepare as the following table.

Components	Volume
Mixture from step 2	15 µL
Oligo $(dT)_{23}VN$ (50 µM) or Random Primers (50 µM) or Gene Specific Primers (2 µM)	1 µL
5×HyperScript™ IV control Mix	4 µL

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

Temperature	Time
25°C*a	10 min
50–55°C	10 min（for target length≤10 kb） 20 min (for target length＞10kb)
80°C	10 min
*Note: *a. This step is to be set up only when you are taking Random Primers. For Oligo(dT)23VN or Gene Specific Primers, this step is not necessary.	

The products can be used immediately in subsequent PCR or qPCR reactions, or they can be stored for short-term storage at -20°C, for long-term storage, please store at -80°C and avoid repeating freeze-thaw cycles.

[Note 1] If you need PCR to amplify some long 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 minutes to remove RNA.

[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 not exceed 1/10 of the PCR reaction volume.

Primer selection

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. When the templates have complex secondary structures or high GC contents, and the usage of Oligo (dT)$_{23}$VN or Gene Specific Primers can't lead to ideal results, Random Primers can be used.
4. Gene Specific Primer is the most specific primer. 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.

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.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com