HyperScript™ IV First-Strand cDNA Synthesis Kit

Product

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).

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

Features

Features of HyperScript™ IV Reverse Transcriptase:

- Significantly improved resistance to a variety of inhibitors that may interfere with cDNA synthesis
- Robust and specific cDNA synthesis for a wide range of samples
- Faster reverse transcription speed and reduced incubation time from >50 minutes to 10 minutes
- Improved processivity compared to third-generation reverse transcriptase

Components and storage

Components	20 rxns (20 µL reaction)	50 rxns (20 µL reaction)	100 rxns (20 µL reaction)
HyperScript™ IV Reverse Transcriptase (200 U/µL)	20 µL	50 µL	100 µL
5x HSIV 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 1mL

Random Primer (50 μM)	20 μL	50 μL	100 μL
Oligo (dT) $_{23}$VN (50 μM)	20 μL	50 μL	100 μL
Store the components at -20℃ for 2 years.			

Protocol

First-strand cDNA Synthesis

1. RNA denaturation, prepare the mix as the following table 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

Mix gently and briefly centrifuge the components.

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

2. Centrifuge briefly, prepare the reverse transcription reaction system as the following table:

Components	Volume
Mixture from step 1	14 μL
5x HSIV Buffer	4 μL
RNase Inhibitor, Murine (40 U/μL)	1 μL
HyperScript™ IV Reverse Transcriptase (200 U/μL)	1 μL

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

Temperature	Time
25℃[*a]	10 min
50–55°C	10 min（for target length≤10 kb） 20 min (for target length＞10kb)

80°C	10 min

**Notes：*

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 reverse transcription products can be used immediately in subsequent PCR or qPCR reactions, or you can store at -20°C for a short time, for long term 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 not exceed 1/10 of the PCR total volume.

Primer selection

1. If 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 Primer 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 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.
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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