HyperScript™ IV Reverse Transcriptase

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).

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

Protocol

First-strand cDNA Synthesis

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

Components	Volume
50 μM oligo(dT)$_{20}$, or 50 μM Random Primers, or 2 μM gene-specific primer (GSP)	1 μL
10 pg - 5 μg total RNA or 10 pg-500 ng 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.

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	14 μL
5x HSIV Buffer	4 μL
RNase Inhibitor, Murine (40 U/μL)	1 μL
HyperScript™ IV Reverse Transcriptase (200 U/μL)	1 μL
Note 1: The catalog number of RNase Inhibitor, Murine is K1046.	

Mix gently and centrifuge briefly.

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 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.

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.

Notes

1. As a recommended starting point for PCR, reverse transcription reaction (cDNA) should compose 10% of the total reaction volume.
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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