HyperScript™ Reverse Transcriptase Protocol

1. Introduction

HyperScript™ Reverse Transcriptase is a new enzyme obtained through genetic engineering based on M-MLV Reverse Transcriptase. In comparison, HyperScript™ Reverse Transcriptase reduces RNase H activity and increases thermal stability. HyperScript™ Reverse Transcriptase can withstand higher reaction temperatures and is suitable for reverse transcription of RNA templates with complex secondary structures. In addition, HyperScript™ Reverse Transcriptase enhances the affinity to the template, suitable for the reverse transcription of a small number of templates and low copy genes, and can generate cDNA up to 12.3 kb.

HyperScript™ Reverse Transcriptase is generally used to achieve reverse transcription of RNA into cDNA for subsequent qPCR experiments or other experiments.

2. Components and Storage

Components	2000 U	10000 U	40000 U
HyperScript™ Reverse Transcriptase (200 U/μL)	10 μL	50 μL	4x50 μL
5x First-Strand Buffer	500 μL	500 μL	2x500 μL

Store the components at -20°C.

3. Protocol

3.1. First-strand cDNA Synthesis

1. RNA denaturation (Optional step. RNA denaturation helps to open the secondary structure and can greatly increase the amounts of first strand cDNA product). Prepare the following mixed solution (10 mM dNTP Mixture, Cat. K1041):

Component	Volume
Oligo(dT)$_{12-18}$(500 μg/mL) or 50-250 ng Random Primers or 2 pmole gene-specific primer (GSP)	1 μL
1 ng to 5 μg total RNA or 1-500 ng of mRNA	X μL
10 mM dNTP Mixture	1 μL

RNase free ddH_2O	to 12 μL

Heat mixture at 65°C for 5 min, and then quickly cool on ice for 2 min.

2. Centrifuge immediately and prepare a reverse transcription reaction system.

Component	Volume
Products of Step 1	12 μL
5x First-Strand Buffer	4 μL
RNase free ddH2O	2 μL
RNase Inhibitor, Murine(40 U/μL) (optional)*	1 μL

✧ **RNase Inhibitor, Murine (40 U/μL) (Cat. No. K1046) is required if using <50 ng starting RNA.**

3. Gently mix the above reactions. If you are using Random Primers, incubate at 25°C for 2 min.
4. Add 1 μL (200U) of HyperScript™ Reverse Transcriptase to the reaction tube and mix gently with a pipette (If your RNA template is less than 1 ng, add HyperScript™ Reverse Transcriptase 0.25 μL and use RNase free ddH_2O to complete the final reaction volume to 20 μL).
5. Incubate at 42-50°C for 50 min.

✧ Even though the template RNA has a strong secondary structure, HyperScript™ Reverse Transcriptase has powerful amplification capabilities, so it is generally recommended to perform the reaction at 42°C. However, in the RT-PCR experiment, if the reverse primer of PCR is used as a reverse transcription primer, non-specific products will be incorrectly amplified. In this case, it is recommended to perform the reaction at 45-50 °C.

6. Inactivate and stop the reverse transcription reaction by heating at 70°C for 15 min.
7. The obtained reverse transcription products can be used immediately for subsequent PCR or qPCR reactions, and can also be stored at -20°C for short-term storage and long-term storage at -80°C , and avoid repeated freeze-thaw.

3.2. PCR

✧ The following steps are perform a PCR reaction with Taq DNA polymerase using the first-strand cDNA as a template. The concentration of Mg^{2+} in the reaction system depends on the template and primers. Generally, only 10% of the volume of the first-strand cDNA reaction solution is used for PCR. A larger volume of template does not increase amplification and may result in

reduced PCR products.

1. Prepare a 50 μL reaction system by referring to the following table (Taq DNA Polymerase, Cat. K1035)

Component	Volume
10X PCR Buffer	5 μL
50 mM $MgCl_2$	1.5 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)	0.4 μL
cDNA from first-strand reaction	2 μL
ddH_2O	to 50 μL

2. Gently mix the above reaction system and centrifuge it instantaneously, and place it in the PCR instrument for PCR reaction.

Temperature	Time	Cycles
94°C	2 min	1
94°C	30 s	
Tm-5°C	30s	15-40
72°C	1min	
72°C	5 min	1
4°C	∞	1