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MorreRT cDNA Synthesis Kit

The MorreRT cDNA Synthesis Kit For Long-Fragment cDNA Amplification

The MorreRT cDNA Synthesis Kit (+gDNA remove mix) is designed for the 1st strand cDNA synthesis with genomic DNA removal treatments. The MorreRT Reverse Transcriptase is a new generation reverse transcriptase optimized from the M-MLV (RNase H-) Reverse Transcriptase. The half-life of MorreRT Reverse Transcriptase at 50°C is > 240 min. Even at 55°C, the MorreRT Reverse Transcriptase can stay stable for a long time, which significantly benefits the transcription of RNA templates with complex secondary structures. In addition, the MorreRT Reverse Transcriptase has a improved template affinity and cDNA synthesis efficiency. It has a good resistance to most RT-PCR inhibitors and is suitable for long-fragment cDNA amplification (as long as 20 kb).

The residual genomic DNA in RNA template can be removed rapidly and completely after a treatment (42°C for 2 min) with the 4× gDNA remove mix. The 10× RT Mix contains an optimized buffer and dNTPs. The MorreRT Reverse Transcriptase Mix contains the MorreRT Reverse Transcriptase and the RNase inhibitor. The Oligo-$(dT)_{23}$ has a better affinity to Ploy A+ RNA than Oligo-$(dT)_{18}$. In addition, random hexamers and gene-specific primers (GSP) are also optional.

Order Information

Product	Cat. No.	Quantity
MorreRT cDNA Synthesis Kit (+ gDNA Remove mix)	MRTK-GR50	50 rxn

Contents of Kits

Component	Amount
RNase free ddH_2O	1mL
4x gDNA remove mix	200 µL
10x MorreRT Buffer with dNTP	100 µL
MorreRT Reverse Transcriptase Mix with RNase inhibitor	100 µL
Oligo dT23 (50uM)	50 µL
Random hexamers (50ng/ul)	50 µL

Storage

All components should be stored at -20°C.

Additional Materials Required

RNase-free microtube (1.5 mL) or PCR tube (0.2 mL).
PCR instrument or water bath.
Ice bath.

Protocol

Note:

1. Use high quality total RNA with high intergrity for reverse transcription.
2. To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.
3. Primer selection (Oligo-dT, Random hexamers, or GSP):

If cDNA product will be used for PCR.

- ✧ For eukaryotic RNA templates, generally, use oligo-dT to obtain the highest yield of full-length cDNA.
- ✧ Use gene-specific primer (GSP) to obtain the highest specificity. However, switch to oligo-dT or random haxamers if GSP fails in the 1st. strand cDNA synthesis.
- ✧ Random hexamers with the lowest specificity can be used for RNA templates, including mRNA, rRNA, and tRNA. Use random hexamers when oligo-dT or GSP fails in cDNA synthesis due to complex secondary structure, high GC content, or prokaryotic RNA template.

If cDNA product will be used for qPCR.

- ✧ Use the mixture of oligo-dT or random hexamers.

1 If cDNA prodcuct will be used for PCR.

1.1 RNA Denaturation*.
Mix the following components in a RNase-free PCR tube, and incubate at 65°C for 5min and then chill on ice immediately for 2 min.

RNase free ddH_2O	to 12 μL
Oligo dT23 (50uM) or Random hexamers (50ng/ul)	1 μL
Total RNA or PolyA+ RNA	10pg - 5μg 10pg- 500ng

* RNA denaturation benifits the cDNA yield. However, for cDNA < 3 kb, please skip the denaturation step.

1.2 Removal of Genomic DNA, and incubate at 42°C for 2 min.

Mixture of Step1.1. (12 μL)	12 μL
4x gDNA remove mix	4 μL

1.3 Mix the following components in a RNase-free PCR tube by gently pipetting.

Mixture of Step 1.2. (16 μL)	16 μL
10x MorreRT Buffer with dNTP	2 μL
MorreRT Reverse Transcriptase Mix with RNase inhibitor	2 μL

1.4 Start the 1st-strand cDNA synthesis.

Temperature	Duration
25°C*	5 min
50°C**	45 min
85°C	5 min

* Only necessary when using random hexamers. Please skip this step when using Oligo dT23 or Gene Specific Primers (GSP).

** For templates with complex secondary structure or high GC-content, the temperature can be increased to 55°C, which will benefit the yield.

1.5 The products can be used for PCR immediately or be stored at -20°C for 6 months. However, it is recommended to store at -80°C and make aliquots to avoid repeated freezing and thawing.

2 If cDNA prodcuct will be used for qPCR.

2.1 Removal of Genomic DNA Mix the following components in a RNase-free microtube by pipetting, and incubate at 42°C for 2 min.

RNase free ddH_2O	to 16 μL
4x gDNA remove mix	4 μL
Oligo dT_{23} (50uM)	1 μL
Random hexamers (50ng/ul)	1 μL
Total RNA or PolyA+ RNA	10pg - 1μg 10pg- 100ng

2.2 Mix the following components in a RNase-free PCR tube:

Mixture of Step 2.1. (16 μL)	16 μL
10x MorreRT Buffer with dNTP	2 μL
MorreRT Reverse Transcriptase Mix with RNase inhibitor	2 μL

2.3 Start the 1st-strand cDNA synthesis.

Temperature	Duration
50°C*	15 min
85°C	2 min

* For templates with complex secondary structure or high GC-content, the temperature can be increased to 55°C, which will benefit the yield.

2.4 The products can be used for PCR immediately or be stored at -20°C for 6 months. However, it is recommended to stored at -80°C and make aliquots to avoid repeated freezing and thawing.