MORREBIO
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MorreRT Reverse Transcriptase

The MorreRT For Long-Fragment cDNA Amplification

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℃ is > 240 min. At 55℃, the MorreRT Reverse Transcriptase can also be stable for a long time, which significantly benifits 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).

Contents of Kits

Component	Volume
MorreRT Reverse Transcriptase (10,000U)	50ul
5× MorreRT Buffer	500 ul

Storage

All components should be stored at -20℃.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTPs into acid-insoluble products in 10 min at 37℃ with Poly(rA)-Oligo (dT) as the template / primer.

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 prodcuct will be used for PCR

- **For eukaryotic RNA tempaltes, 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 lowerst 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 prodcuct 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:

Rnase free ddH_2O	to 13 μl
Oligo dT_{23} (50 ng/μl) or Random hexamers (50ng/μl) or Gene Specific Primers (2μM)	1 μl
Total RNA	10 pg-5 ug
or Poly A^+ RNA	10 pg-500 ng

Incubate at **65**℃ for 5 min and then chill on ice immediately for 2 min.

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

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

Mixture of Step 1.1.	13 μl
5x MorreRT Buffer	4 μl
dNTP Mix (10mM each)	1 μl
MorreRT Reverse Transcriptase	1 μl
Rnase inhibitor (40U/ul)	1 μl

1.3. Start the 1st-strand cDNA synthesis.
Mix the following components in a RNase-free PCR tube:

25℃*	5 min
50℃**	45 min
85℃	5 min

Note: * Only necessary when using random hexamers. Please skip this step when using Oligo dT_{23} or Gene Specific Primers (GSP).
** For templates with complex secondary structure or high GC-content, the temperature can be increased to 55℃, which will benefit the yield.

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

2. If cDNA prodcuct will be used for qPCR

2.1. Mix the following components in a RNase-free

RNase free ddH2O	to 20 μl
5× MorreRT Buffer	4 μl
dNTP Mix (10mM each)	1 μl
MorreRT Reverse Transcriptase	1 μl
RNase inhibitor (40 U/μl)	1 μl
Oligo dT_{23} (50 μM)	1 μl
Random hexamers (50 ng/μl)	1 μl
Total RNA	10 pg-1 μg
or Poly A+ RNA	10 pg-100

2.2. Start the 1st-strand cDNA synthesis.

25℃	5 min
50℃*	15 min
85℃	5 min

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

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