All-in-One cDNA Synthesis SuperMix

Notice!

- **This product is designed for use in real-time quantitative PCR (RT-qPCR).**
- **Digestion of genomic DNA in RNA template before cDNA synthesis is strongly recommended.**

1. General Information

In one tube, the pre-blended 5 × qRT SuperMix contains all the necessary components for reverse transcription of YOUR RNA template. The resulting cDNA product is suitable for use in real-time quantitative RT-PCR (qRT-PCR).

2. Contents

Component	B24403(200 rxn)	B24408(1000 rxn)
5 × qRT SuperMix[a]	400 µL	1 mL × 2
5 × No RT ControlMix[b]	40 µL	200 µL
RNase-free Water	1 mL × 2	1mL × 10

a: contains Buffer, dNTP, Reverse Transcriptase, RNase inhibitor, Random primer/Oligo (dT) primer mix.

b: contains all the components in 5 × qRT SuperMix, except Reverse Transcriptase.

3. Storage and Stability

Store at -20°C for up to 2 years. It is recommended to pre-aliquot the mix into small batches for frequent usage. Product quality is guaranteed under proper storage conditions.

4. Protocol

The following protocol has been optimized for generating first-strand cDNA for use in qRT-PCR.

1. Combine the following components in a tube on ice.

Component	Volume
Total RNA/mRNA	50 ng-5µg/5-500 ng
5 × qRT SuperMix	2 µL
RNase-free Water	Up to 10 µL

***Note*:** Digestion of genomic DNA in RNA template with DNase I beforehand is strongly recommended.

No RT control (optional)

Component	Volume
Total RNA/mRNA	50 ng-5µg/5-500 ng
5× No RT ControlMix	2 µL
RNase-free Water	Up to 10 µL

***Note*:** The reaction is for detection of false positive results by genomic DNA contamination in RNA template.

2. Gently mix tube contents and incubate at 42°C for 15 minutes (for Standard procedure).
3. Incubate tube at 85°C for 2 minutes to inactivate All-in-One Reverse Transcriptase. Chill on ice until contents reach room temperature.
4. Store at -20°C until use.

For Optional procedure:

To obtain cDNA of higher quality, combine RNA template and 5 x qRT SuperMix in a tube on ice, incubate the tube at 25°C for 10 minutes, then at 42°C for 30 minutes for extension, and finally at 85°C for 5 minutes to terminate the reaction.

5. Troubleshooting

Q1: What are the important notes while using this product?

A1: Gently invert the tube upside down several times before use. Do NOT vortex. Brief centrifugation prior to use is recommended.
High-quality RNA template is recommended to ensure successful cDNA synthesis.
To eliminate false positive results by genomic DNA contamination, digestion of template RNA with DNase is strongly recommended.
For RNA templates of complex structure or to achieve higher synthesis efficiency of standard RNA templates, try the Optional procedure as described.

Q2: What's the function of mixing All-in-One Oligo (dT) and Random Primer?

A2: Mixed priming strategy eliminates end-bias. This kit includes an optimized Primer Mix which results in the generation of first-strand cDNAs from an entire transcript without the end-bias observed with typical oligo (dT) or random primers. This mixed primer strategy overcomes variability in real-time PCR gene expression analysis that can result from using different individual primers. (As the figures show on the next page)

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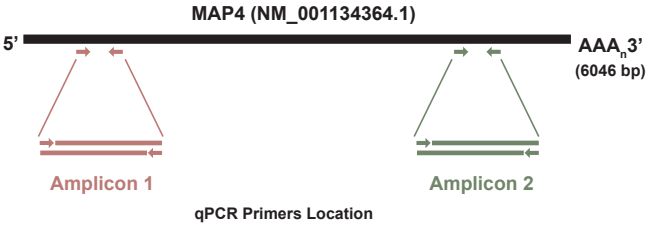
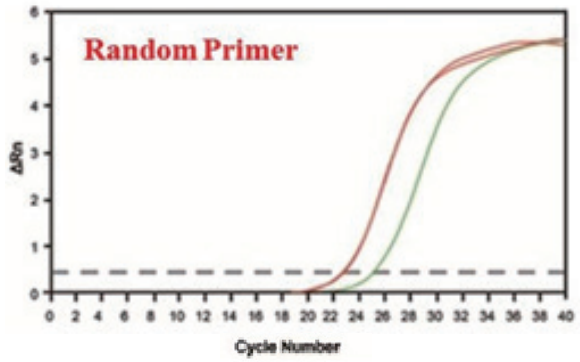
A



Amplicons located near the (A) 5' end (orange) or 3' end (green) of the MAP4 transcript were amplified by real-time PCR using 1μL of each reverse transcription reaction in 20 μL real-time PCR reactions.

As the figures show, the Primer Mix solution greatly reduces bias for sequences near the 5' or 3' ends of cDNAs produced. Microtubule-associated protein 4 (MAP4) mRNA was reverse transcribed from 100 ng of HeLa cell total RNA using (B) Random Primer, (C) Anchored Oligo (dT) Primer, or (D) the Primer Mix.

B



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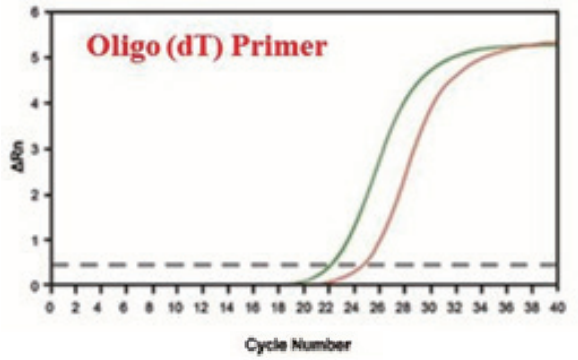

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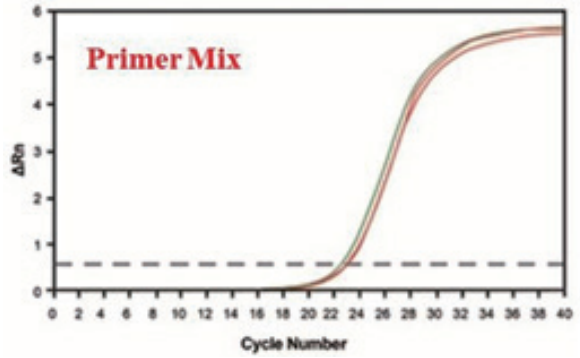

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