

Catalog No.: G209
Product Name: **5X All-in-One Universal RT MasterMix**
Concentration: 5X
Size: 100 rxns

Kit Components:	5X All-in-One Universal RT Plus MasterMix	400 μ l
	Nuclease-free H ₂ O:	1000 μ l

Description: The **5X All-in-One Universal RT MasterMix** is a ready-to-use master mix for first-strand cDNA synthesis. It is in a 5X concentration for enzyme stability and flexible reaction volume. This master mix contains the EasyScript™ Plus Reverse Transcriptase, ribonuclease inhibitor, cDNA synthesis enhancers, dNTPs, and a balanced concentration for oligo(dT) and random primers. It also contains a temperature sensitive DNase for residual genomic DNA (gDNA) removal. The finely-balanced ratio of Oligo (dT)s and Random Primers, ensures the full coverage of the temperate for cDNA synthesis. The high-quality cDNA synthesized from this kit can be used for a wide range of downstream applications, such as gene expression study or other real-time RT-qPCR experiments.

Advantages:

- Convenience: one tube, one reaction, one pipetting for RT reagents.
- Accuracy: ensures sample to sample consistency.
- Capacity: capable for long or complex RNA templates.
- Flexibility: large RNA sample or reaction volume.
- Sensitivity: work as well at low concentration of RNA samples.

Storage: Store at -20°C.

Protocol:

1. Thaw RNA sample and all reagents on ice. Mix each solution completely but gently.
2. Assemble the following components in a tube on ice:

Components	Volume	Final Conc.
Total RNA, or mRNA	Variable	1ng - 2 μ g/rxn 0.1pg - 1ng/rxn
5X RT MasterMix	4 μ l	1X
H ₂ O	Up to 20 μ l	-

3. Mix well but gently and briefly centrifuge the tube at high speed.
4. Incubate the mixture at 37°C for 15 min, followed by 15 minutes incubation at 55°C.
5. Stop the reaction by heating it at 85°C for 10 min.
6. Chill the tube on ice.

Note: Alternatively, use a PCR machine to perform the cDNA synthesis with the following temperature cycle:
37°C, 15 min; 55°C for 15 min; 80°C for 10 min; and 4°C for any desired time.

7. Collect the mixture by briefly centrifugation at high speed. The cDNA is now ready for immediate downstream applications or leave at -20°C for long-term storage.

Extra Notes:

- Both poly(A) mRNA and total RNA can be used for first-strand cDNA synthesis, although poly(A) mRNA may give higher yield and improved purity of final products.
- For longer transcripts >9 kb, yield can be increased by incubating at 50-55°C for 30-50 minutes.