Applied Biological Materials Inc.
Tel: 1-866-757-2414
Email: info@abmGood.com
Website: www.abmGood.com

5X All-In-One RT MasterMix (with AccuRT Genomic DNA Removal Kit)

Cat. No. G492

Store at -20°C

Part No.	Components	Volume
G488-1	AccuRT Reaction Mix (4X)	200 µl
G488-2	AccuRT Reaction Stopper (5X)	200 µl
G490	5X All-In-One RT MasterMix	400 µl
RT-0	Nuclease-free H_2O	1 ml
	Size	**100 rxns**

Product Description

abm's 5X All-In-One RT MasterMix (with AccuRT Genomic DNA Removal Kit) provides a convenient and highly efficient method for first-strand cDNA synthesis with an additional genomic DNA removal step now included. The presence of contaminating genomic DNA (gDNA) in RNA preparations is often a significant problem for downstream applications, leading to false-positive signals and misinterpretation of gene expression levels. Effective elimination of gDNA is therefore the most reliable method to ensure accurate experimental results.

The AccuRT Genomic DNA Removal Kit provided will effectively remove contaminating gDNA from the sample in under 10 minutes, without any heating or organic extraction steps which can result in damage to the RNA template. The treated gDNA-free RNA can then be directly reverse-transcribed into cDNA using the 5X All-In-One RT MasterMix. The 5X All-In-One RT MasterMix is a ready-to-use formulation of all the reagents necessary for first-strand cDNA synthesis. Coupled together, this complete system provides the ultimate convenience in generating high-quality cDNA suitable for a wide range of downstream applications.

Note: Upon completion of the first-strand cDNA synthesis, the cDNA product can be directly applied as a template in a standard PCR/qPCR.

Storage Conditions

Store all components at -20°C in a non-defrost cycle freezer. All components are stable for 1 year from the date of shipment when stored and handled correctly.

Protocol

Both gDNA removal and reverse transcription reactions should be assembled in a RNasefree environment. The use of "clean", automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended. Keep the RNA on ice to minimize RNA degradation.

1. Thaw template RNA on ice. Thaw all reagents at room temperature and spin briefly to collect residual liquid from the sides of the tubes. Keep the thawed reagents on ice.
2. Prepare the following reactions for gDNA removal and subsequent reverse transcription on ice:

Components	Volume
RNA Template	Up to 2 µg
AccuRT Reaction Mix (4X)	2 µl
Nuclease-free H_2O	Up to a total volume of 8 µl
Incubate at 42°C for 2 minutes or room temperature for 5 minutes, then add the following to the tube:	
AccuRT Reaction Stopper (5X)	2 µl
The purified RNA is ready for first-strand cDNA synthesis. Set-up the reverse transcription reaction by adding the components below into the tube:	
5X All-In-One RT MasterMix	4 µl
Nuclease-free H_2O	6 µl
Total Reaction Volume	20 µl
Incubate at 25°C for 10 minutes, then incubate at 42°C for another 15 minutes (if downstream application is qPCR) or 50 minutes (for PCR). Inactivate the reaction at 85°C for 5 minutes. Chill on ice.	

3. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or long-term storage at -20°C.

General Notes

To remove the RNA complementary to cDNA, add 1 µl (2 U) of *E. coli* RNase H (Cat. No. **E018**) and incubate at 37°C for 20 minutes.