PRODUCT INFORMATION

5X All-In-One RT MasterMix (with Cell Lysis Kit)

Product name: **5X All-In-One RT MasterMix (with Cell Lysis Kit)**
Catalogue Number: **HRT100-20L**
Size: 100 Rxns
Storage: Store all components at -20°C in a non-defrosting freezer. All components are stable for 1 year from the date of shipment when stored and handled correctly.

Components	Volume
Lysis Solution	1.25ml x 2
Stop Solution	300 µl
Protease	50 µl
Protease Inhibitor	50 µl
5X All-In-One RT MasterMix	400 µl
Nuclease-free H2O	2 x 1ml
Size	**25 Lysis Preps, 100 Reactions**

Product Description

BBI 5X All-In-One RT MasterMix (with ExCellenCT Lysis Kit) offers a quick, simple and robust method to prepare template for fi rst-strand cDNA synthesis directly from cultured cells, enabling reverse transcription of lysates from 10-105 cultured cells without time-consuming and hazardous-chemicals-involved RNA extraction and purification steps. The kit includes reagents for cell lysis as well as gDNA removal. 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. The lysis procedure simultaneously eliminates genomic DNA effectively in 12 minutes, without compromising RNA quality, and therefore ensures consistent, reproducible, and accurate results with 10-105 cells.

The lysate 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 fi rst-strand cDNA synthesis, including proprietary ReverseTranscriptase, Ribonuclease Inhibitor, dNTPs and a finelybalanced ratio of Oligo (dT)s and Random Primers. Coupled together, this complete system provides the ultimate convenience in generating high-quality cDNA directly from 10-10^5 cultured cells, suitable for a wide range of downstream applications.

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

Protocol

Both cell lysis and reverse transcription reactions should be assembled in a RNase-free environment. The use of “clean”, automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended. Keep the cell lysates on ice to minimize RNA degradation.

1. Thaw Lysis Solution and Stop Solution. Homogenize each solution gently but thoroughly.
2. Prepare the following reactions for cell lysis:

Components	Volume
10-10^5 cells	5 µl
Protease	1 µl
Lysis Solution	50 µl
Mix content by pipetting 35 µl of the mixture up and down 5 times and avoid creating bubbles. *Incubate at 37°C for 10 minutes, then add the following to the tube:*	
Protease Inhibitor	1 µl
Stop Solution	5 µl
Mix content by pipetting 35 µl of the mixture up and down 5 times. *Incubate at room temperature for 2 minutes, then store the lysate on ice.*	

3. The lysate is ready for fi rst-strand cDNA synthesis. Set up the reverse transcription reaction by adding the components below:

Components	Reaction Volume	
	10 µl	20 µl
5X All-In-One RT MasterMix	2 µl	4 µl
Cell Lysate (from previous step)	2 µl	4 µl
Nuclease-free H2O	to 10 µl	to 20 µl
Incubate at 25°C for 10 minutes, then incubate at 42°C for another 30 minutes. *Inactivate the reaction at 85°C for 5 minutes. Chill on ice.*		

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

General Notes

- Minimize RNA degradation by keeping cells in PBS on ice before starting the cell lysis procedure.
- Do not vortex Stop Solution.
- Lysis Solution and Stop Solution must be at room temperature before proceeding to the lysis procedure.
- As cells settle quickly, thoroughly resuspend cells before withdrawing cell solution samples.
- (Optional) If setting up multiple reactions, prepare Protease / Lysis Solution premix for the

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number of reactions required, and then mix the premix solution with 5 µl of 10-105 cells.

- (Optional) If setting up multiple reactions, prepare Protease Inhibitor / Stop Solution premix for the number of reactions required, and then mix the premix solution with the lysis reaction.
- As RNAs are poor templates for DNA polymerase, a Ct difference of 8-12 would be expected in qPCR between reactions containing RTase and those with no RTase.
- Lysates can be safely stored on ice for up to 1 hour after lysis. Alternatively, lysates can be stored at -80oC for a short period of time with a maximum of 1 freeze / thaw cycle. We highly recommend to use the lysates in downstream applications immediately after the 2 minutes termination.

For laboratory research only. Not for clinical applications.
For technical questions, please email us at tech@biobasic.com
Or visit our website at www. biobasic.com

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