

Evo™ RT Mastermix (Cell Lysis)

(Cat# M1172-100; 5X All-In-One RT Mastermix; Store at -20°C)

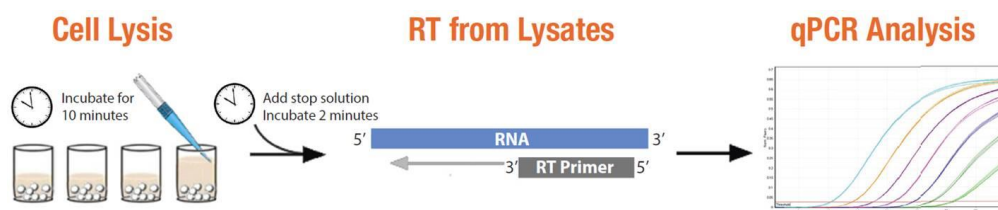
I. Introduction:

Evo™ RT Mastermix (with cell lysis) is a 5X All-In-One RT Mastermix (with Cell Lysis Kit) that offers a quick, simple and robust method to prepare template for first-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 genomic DNA (gDNA) removal. The presence of contaminating 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 min, 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 first-strand cDNA synthesis, including Biovision's proprietary Evo™ Reverse Transcriptase (Evo™ RTase), RNaseOFF Ribonuclease Inhibitor, dNTPs and a finely balanced ratio of Oligo(dT)s and Random Primers. Coupled together, this complete system provides the ultimate convenience in generating high-quality cDNA directly from 10-10⁵ cultured cells, 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.

BioVision's Evo™ RT Mastermix (with cell lysis) is only intended for quick RNA extraction from cultured cells for two-steps RT PCR or two-steps QRT PCR gene expression analysis.



II. Application:

- Gene expression studies

III. Package Contents (Evo™ RT Mastermix (Cell Lysis)):

Components	M1172-100 (25 lysis preps/100 X 20 µl RT rxns)	Part Number
Lysis Solution	1.25 ml X 2	M1172-XX-1
Stop Solution	300 µl	M1172-XX-2
Protease	50 µl	M1172-XX-3
Protease Inhibitor	50 µl	M1172-XX-4
5X All-In-One RT Mastermix	400 µl	M1172-XX-5
Nuclease-free H ₂ O	2 X 1 ml	M1172-XX-6

IV. User Supplied Reagents and Equipment:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- RNA Template

V. Shipment and Storage:

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly. Avoid repeated freeze-thaw cycles to retain maximum performance. Briefly centrifuge small vials prior to opening.

VI. 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 min, 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 min, then store the lysate on ice

3. The lysate is ready for first-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 μ l	4 μ l
Cell Lysate (from previous step)	2 μ l	4 μ l
Water, Nuclease-free	to 10 μ l	to 20 μ l

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

VII. 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 number of reactions required, and then mix the premix solution with 5 μ l of 10^5 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 -80°C 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 min termination.

IX. Related Products:

BV Product Name	BV Cat. No.
Two Step RT PCR Kits	M1160-M1161
One Step RT PCR Kits	M1162-M1163
First-Strand cDNA Synthesis Kits	M1164-M1167
First-Strand cDNA Synthesis Supermixes	M1167-M1169
All-In-One RT Mastermixes	M1170-M1172
Reverse Transcriptases	M1173-M1174
One Step Jade™ QRT PCR Kits	M1175-M1182
One Step Taqman QRT PCR Kits	M1183-M1190

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