Rev. 0

Direct Lysis Buffer for RT-qPCR (DLB-RT)

Catalog #7218
100 reactions

Product Description

Gene expression analysis by reverse transcription quantitative PCR (RT-qPCR) typically requires RNA extraction using organic reagents or silica column-based kits. These methods involve multiple processing steps, are time-consuming, and often result in significant RNA loss during purification. This can be particularly problematic for low-input samples, where RNA recovery may be insufficient for reliable downstream analysis.

The ScienCell Direct Lysis Buffer for RT-qPCR (DLB-RT) is a ready-to-use, optimized for rapid cell lysis and direct input into cDNA synthesis reactions. DLB-RT efficiently disrupts cultured mammalian cells in both adherent and suspension formats, releasing total RNA in a form compatible with downstream reverse transcription and qPCR amplification. The streamlined one-step workflow eliminates RNA purification entirely, reducing total processing time to under 15 minutes while minimizing RNA loss and degradation risk associated with multi-step handling.

DLB-RT is suitable for 100 to 50,000 cells per reaction, depending on the cell type.

Kit Components

Cat #	Component	Quantity	Storage
7218a	Direct Lysis Buffer	5 mL	4°C
7218b	RNase Inhibitor	1 vial	-20°C

Additional Materials Required (Not Included)

1.1. PBS (phosphate-buffered saline), sterile
1.2. Microcentrifuge (suspension cell protocol)
1.3. Pipettes and RNase-free tips
1.4. Reverse transcriptase enzyme and RT reaction components
1.5. PCR-grade water
1.6. qPCR master mix and validated primers/probes
1.7. 96-well PCR plates or strip tubes; qPCR instrument

Quality Control

Each lot of Direct Lysis Buffer for RT-qPCR is tested for the following criteria prior to distribution: undetectable RNase activity and DNase activity; and functional RT-qPCR performance with Ct values ≤27 for GAPDH using 1,000 HUVEC cells.

Product Use

DLB-RT is designed for direct lysis of cultured mammalian cells for input into cDNA synthesis reactions, enabling RT-qPCR-based gene expression analysis without RNA purification. It is

compatible with standard one-step and two-step RT-qPCR workflows. Suitable for adherent and suspension mammalian cell cultures. This product is for Research Use Only. It is not approved for human or animal diagnostic procedures or *in vitro* diagnostic applications.

Shipping and Storage
The product is shipped on dry ice. Upon receipt, store the components at -20°C. Once thawed, store Cat.#7218a at 4°C.

Procedures

Cell Plating & Preparation:

A. **Cell Density:** Plate the optimal number of cells/well in a 96-well tissue culture plate.
B. **Buffer preparation:** Add 0.5 U of RNase Inhibitor (cat#7218b) per µL of lysis buffer before use. For example, for one 96-well plate, mix 5 mL of lysis buffer with 2,500 U of RNase inhibitor.

Note: *Optimal cell number may vary depending on the cell type and target gene expression level. For most cultured mammalian cell types, 100–10,000 cells per reaction provide excellent results. Users are encouraged to empirically determine the optimal cell input for their specific application.*

Lysis Protocol for Adherent or Suspension Cells

For Adherent cells	For Suspension cells
1. Remove culture medium. 2. Wash cells once with PBS. 3. Remove residual PBS; tap plate dry. 4. Add 50 µL Direct Lysis Buffer per well. 5. Pipette up and down 5 times. 6. Shake/incubate 10 min at room temperature. 7. Transfer 5 µL lysate to a 20 µL cDNA synthesis reaction. 8. Perform reverse transcription.	1. Pellet cells by centrifugation. 2. Remove the culture medium. 3. Add PBS to the pellet and resuspend. 4. Pellet cells by centrifugation. 5. Remove residual PBS. 6. Add 50 µL Direct Lysis Buffer per well. 7. Pipette up and down 5 times. 8. Incubate 10 min at room temperature. 9. Transfer 5 µL lysate to a 20 µL cDNA synthesis reaction. 10. Perform reverse transcription

Note: *Proceed with reverse transcription immediately after lysis to ensure optimal RNA integrity and performance. Cell lysate can be stored at* ***-80°C*** *for longer storage.*

Technical Notes

1. Cell input range per well (96 well plate): 100–50,000 cells per reaction. Optimize for each cell type and target gene.
2. Genomic DNA (gDNA) is co-extracted with RNA. DLB-RT does not include a DNase treatment step. Use intron-spanning primers or a –RT control to monitor gDNA amplification, or incorporate a compatible DNase treatment step during cDNA synthesis.
3. A single PBS wash is recommended for prior to lysis to remove residual culture medium components that may inhibit downstream reactions.
4. DLB-RT has been validated with standard commercial reverse transcriptase enzymes.

Troubleshooting Guide

Observation	Possible Cause	Recommended Action
Low or no amplification (high Ct or no Ct).	Insufficient cell number, low target gene expression, or incomplete cell lysis.	Increase cell number (up to 50,000/rxn); extend lysis to 10 min; verify gene expression level in cell type.
High Ct variability between replicates.	Inconsistent pipetting or incomplete cell resuspension.	Pipette up and down at least 5 times; use calibrated pipettes; ensure complete PBS removal before lysis.
Non-specific amplification or primer-dimer.	Genomic DNA (gDNA) co-amplification.	Use intron-spanning primers; include a –RT control; incorporate optional DNase treatment during cDNA synthesis.
No amplification in any condition.	Inhibitors in lysate; failed reverse transcription; degraded RNA.	Verify RT reagent compatibility; process lysate immediately after lysis; check RT enzyme and reagents.
Buffer appears cloudy or precipitated.	Cold-induced precipitation of detergent component.	Warm to room temperature and mix gently before use; do not use if particulates persist.
High lysate viscosity or excessive non-specific amplification.	Overloud cell input (50,000 cells limit), resulting in a high concentration of released genomic DNA (gDNA), that increase lysate viscosity and interfere with accurate pipetting and PCR specificity.	Add another 50 µL lysis buffer. Mix the lysate thoroughly to ensure sample homogeneity and improve PCR performance.