TransScript® Uni Cells to CT 1-Step Probe kit

Please read the manual carefully before use.

Cat. No. AC321

Version No. Version 1.0

Storage: at -18˚C or below for two years

Description

This product features a specialized lysis buffer to disrupt cells, allowing direct analysis of RNA expression in the same reaction system—without lysate purification—while also removing genomic DNA from RNA templates.

Features

- Simple Operation: Lysates are obtained in just 5 minutes and can be directly used for expression analysis without purification. Compatible with highly sensitive qRT-PCR reagents that simultaneously remove genomic DNA, offer high specificity, and support multiplex one-step reactions, ensuring a streamlined workflow.
- Flexible Cell Inputs: Delivers stable amplification across a broad range of cell inputs (10–10^6 cells).
- High Inhibitor tolerance: The qRT system is compatible with input of 6–8 µL lysates.

Kit Contents

Component	AC321-01 (100 rxns)
C to C Lysis Buffer	5×1 ml
5×*TransScript*® II Multiplex Probe One-Step qRT-PCR SuperMix UDG(ROX)	2×1 ml
RNase-free Water	5 ml

Procedures

Cell Lysis

a. Adherent Cells in 48-, 96-, or 384-Well Plates

Cell Number and Buffer Volume for Different Plate Formats

Plate Format	Recommended Cell Number per Well	PBS Volume per Well	C to C Lysis Buffer Volume per Well
384-well	1.25×10^2-1×10^4	25 µl	12.5 µl
96-well	5×10^2-5×10^4	100 µl	50 µl
48-well	1×10^3-1×10^5	250 µl	100 µl

Protocol for Adherent Cell Lysis (96-Well Plate Example)

1. Cell Preparation: Seed and culture cells in a 96-well plate to reach a density of 5×10^2–5×10^4 cells/well at harvest.
2. Remove the medium completely using a pipette.
3. Wash with PBS: Add 100 µL of ice-cold PBS per well, then remove the PBS thoroughly.
4. Lysis: Add 50 µL of C to C Lysis Buffer per well and gently pipette up and down 5–10 times to mix. Incubate at room temperature (22–25°C) for 5 minutes.

b. Protocol for Other Plate Formats (Adherent/Suspension Cells)

1. Cell Preparation: For adherent cells, detach cells using standard passaging methods to obtain a single-cell suspension; For suspension cells, proceed directly to Step 2.
2. Cell Counting & Washing: Count cells, then centrifuge at 500 × g, 5 min, 2–8°C. Discard supernatant.
3. Resuspend pellet in 1 mL ice-cold PBS, centrifuge again (500 × g, 5 min, 2–8°C), and carefully remove supernatant.
4. Cell Resuspension: Adjust cell density to 2–2 × 10^5 cells/µL using ice-cold PBS.
5. Lysis: Transfer 5 µL of cell suspension (containing 10–10^6 cells) to a tube. Add the recommended volume of C to C Lysis Buffer (see table below), pipette mix 5–10 times, and incubate at RT (22–25°C) for 5 min.

Recommended C to C Lysis Buffer Volumes for Different Cell Inputs

Cell Number	C to C Lysis Buffer
10^5-10^6	200 µl
10^3-10^5	50 µl
10-10^3	25 µl

The prepared lysate can be directly used for downstream qRT-PCR reactions or stored at -80°C for up to 1 month if not used immediately.

Recommended qPCR Reaction Components and Conditions (20 µl)

Component	Volume (RT Reaction)	Volume (No RT Control)(Optional)
Cell Lysate	6 µl	6 µl
5×*TransScript*® II Multiplex Probe One-Step qRT-PCR SuperMix UDG(ROX)	4 µl	-
Primer-Probe Mix	Variable	Variable
5×No RT SuperMix UDG(ROX)		4 µl
RNase-free Water	Variable	Variable
Total volume	20 µl	20 µl

Thermal cycling conditions

50°C 5 min
94°C 30 sec
94°C 5 sec
60°C 30 sec ★) 40-45 cycles

Note

For best results, use the recommended cell input range. Higher cell numbers may cause reaction inhibition.

For research use only, not for clinical diagnosis.

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