TransScript® -Uni Cell to cDNA Synthesis SuperMix for qPCR

Cat. No. AC301

Storage: -20°C for two years

Description

TransScript®-Uni Cell to cDNA Synthesis SuperMix for qPCR uses an unique lysis buffer to lyse cells. The resulting lysate (without purification) can be directly used as template for reverse transcription. Unique genomic DNA remover is combined with *TransScript*®-Uni RT/RI Enzyme Mix to achieve simultaneous genomic DNA removal and cDNA synthesis in one tube. This kit is suitable to generate qPCR-ready cDNA directly from cells.

Application

Multiple copy and low copy gene detection

Kit Contents

Component	AC301-01 (25 rxns)
C to C Lysis Buffer	2×1.25 ml
TransScript®-Uni RT/RI Enzyme Mix	12.5 µl
gDNA Remover	12.5 µl
2×TS-Uni Reaction Mix	250 µl
Oligo(dT)/RP Mix	25 µl
RNase-free Water	250 µl

Procedures

Cell Lysis

a. Adherent cells grown in 48-, 96- or 384-well plates

Cell number and buffer volume for different well plates

Plate format	Harvested cells	PBS	C to C Lysis Buffer
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

Adherent cells grown in 96-well plate (example)

1. Inoculate cells in 96-well plate and grow them. Make sure that each well contains 5×10^2-5×10^4 cells.
2. Aspirate and discard the culture medium from the wells.
3. Add 100 µl of cold PBS to each well, and aspirate the PBS from the well. Remove as much PBS as possible without disturbing the cells.
4. Add 50 µl of C to C Lysis Buffer to each well, incubate at room temperature (22°C-25°C) for 5 minutes.
5. Mix by pipetting up and down, and then transfer the lysis into a microcentrifuge tube. Incubate at 75°C for 5 minutes, then place the tube on ice.

b. Cells grown in other format plates

1. For adherent cells, detach cells using the subculturing method routinely used in your laboratory for the cell type. For suspension cells, skip step 1.
2. Count cells, centrifuge at 2-8°C, 500×g for 5 minutes, gently discard the supernatant.
3. Resuspend cells with 1 ml of cold PBS, centrifuge at 2-8°C, 500×g for 5 minutes, gently discard the supernatant.
4. Resuspend cells with proper volume of cold PBS to allow cell density at 5×10^2-2.5×10^5/µl.

5. Add 2 µl of cells into 100 µl of C to C Lysis Buffer, incubate at room temperature (22°C-25°C) for 5 minutes.
6. Incubate at 75°C for 5 minutes, and then place the tube on ice.
(cell lysate from this step can be directly used for reverse transcription; or lysates can be stored at -20°C for one week or at -80°C for 2 months.)

First-strand cDNA synthesis and gDNA removal

1. Reaction Components

Component	Volume
Cell Lysate	2 µl
Oligo(dT)/RP Mix	1 µl
2×TS-Uni Reaction Mix	10 µl
gDNA Remover	0.5 µl
TransScript®-Uni RT/RI Enzyme Mix	0.5 µl
RNase-free Water	to 20 µl

2. Gently mix and incubate at 42°C for 15 minutes.
3. Incubate at 85°C for 5 seconds to inactivate *TransScript*®-Uni RT/RI Enzyme Mix and gDNA Remover.

qPCR Reaction components (20 µl reaction volume)

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
2×*TransStart*® Top/Tip Green qPCR SuperMix	10 µl	1×
Passive Reference Dye (50×) (optional)	0.4 µl	1×
Nuclease-free Water	Variable	-
Total Volume	20 µl	-

Thermal cycling conditions (three-step)

94°C 30 sec
94°C 5 sec
50-60°C 15sec★ } 40-50 cycles
72°C 10sec★
Dissociation Stage

Thermal cycling conditions (two-step)

94°C 30 sec
94°C 5 sec
60°C 30 sec★ } 40-50 cycles
Dissociation Stage

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

★ For ABI Prism7700/7900, set time to 30 seconds.
★ For ABI Prism7000/7300, set time to 31 seconds.
★ For ABI Prism7500, set time to 34 seconds.
★ For ABI ViiA7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.
Three-step qPCR is more suitable for higher sensitivity assay.

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