FlysisAmp Cells-to-CT 1-Step Probe Kit

CL131

Vazyme

Instruction for Use

Version 24.1

Vazyme

Contents

01/Product Description

The FlysisAmp Cells-to-CT 1-Step Probe Kit is a complete kit that quickly and efficiently goes from "cells" to "amplification results" and is compatible with 10 - 10^6 cultured cells. Genomic DNA elimination and RNA acquisition can be completed directly in the cell culture plate. With the effective, stable, and specific probe-based RT-qPCR mix for multiplex amplification, gene expression results can be obtained from cells as fast as 37 min. This kit eliminates the complicated process of traditional extraction, improves efficiency and reduces sample loss in the purification process. This kit is compatible with a wide range of cell types (adherent or suspension cells) and cell quantities, and can also solve the problem of unsatisfactory extraction results with low cell numbers in traditional extraction methods. This kit can be used for high-throughput (96-/384-well cell culture plate) cellular RNA expression analysis.

02/Components

Components		CL131-01 100 rxns (50 µl/rxn)	CL131-02 500 rxns (50 µl/rxn)
☐	FlysisAmp Cells Lysis Buffer[a]	5 ml	25 ml
■	DNase I	200 µl	1 ml
■	Enhancer Solution	200 µl	1 ml
■	FlysisAmp Cells Stop Buffer	500 µl	2 × 1.25 ml
☐	5 × 1-Step RT-qPCR Mix (ROX)[b]	2 × 1 ml	10 ml

a. FlysisAmp Cells Lysis Buffer can be stored stably for 1 year at 2 ~ 8°C after thawing.

b. It contains dNTP Mix, Mg^{2+}, Taq Pro DNA Polymerase and Specific ROX Reference Dye, etc. It should be stored away from light after unpacking.

03/Storage

Store at -30 ~ -15°C and transport at ≤0°C.

04/Applications

It is applicabe for gene expression analysis from 10 - 10^6 cultured cells (adherent or suspension cells).

05/Self-prepared Materials

1 × PBS buffer, RNase-free ddH_2O, 1.5 ml RNase-free centrifuge tube, RNase-free pipette tip, 0.2 ml RNase-free eight-tube PCR strip/PCR tube, etc.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. Before use, please check whether there is any ice in the reagent. If any component freezes, place it at room temperature to dissolve, and mix well before use.

2. If there are too many cells during the lysis process, it may lead to incomplete lysis or inhibit subsequent reverse transcription and qPCR amplification. Therefore, before operation, adherent cells need to select the corresponding volume of lysis working solution according to the number of plate wells; suspension cells need to be estimated number of cells to select the corresponding volume of lysis working solution.
3. When using fresh samples, if they cannot be lysed in time, wash the cells once with pre-cooled PBS and store them at -85 ~ -65°C, and avoid repeated freezing and thawing. To avoid RNA degradation, cells should be processed and stored as quickly as possible.
4. Gently pipette 8 - 10 times to mix well. Avoid air bubbles caused by vigorous shaking.
5. When using this kit, please wear lab coats, disposable latex gloves, disposable masks, and use RNase-free consumables to avoid RNase contamination.
6. The lysis process is performed in an RNase-free environment; unless otherwise specified, operations are performed at room temperature (15 ~ 25°C).
7. A microplate shaker can be used to shake at 600 rpm for 30 sec instead of pipetting to mix.

07/Mechanism & Workflow

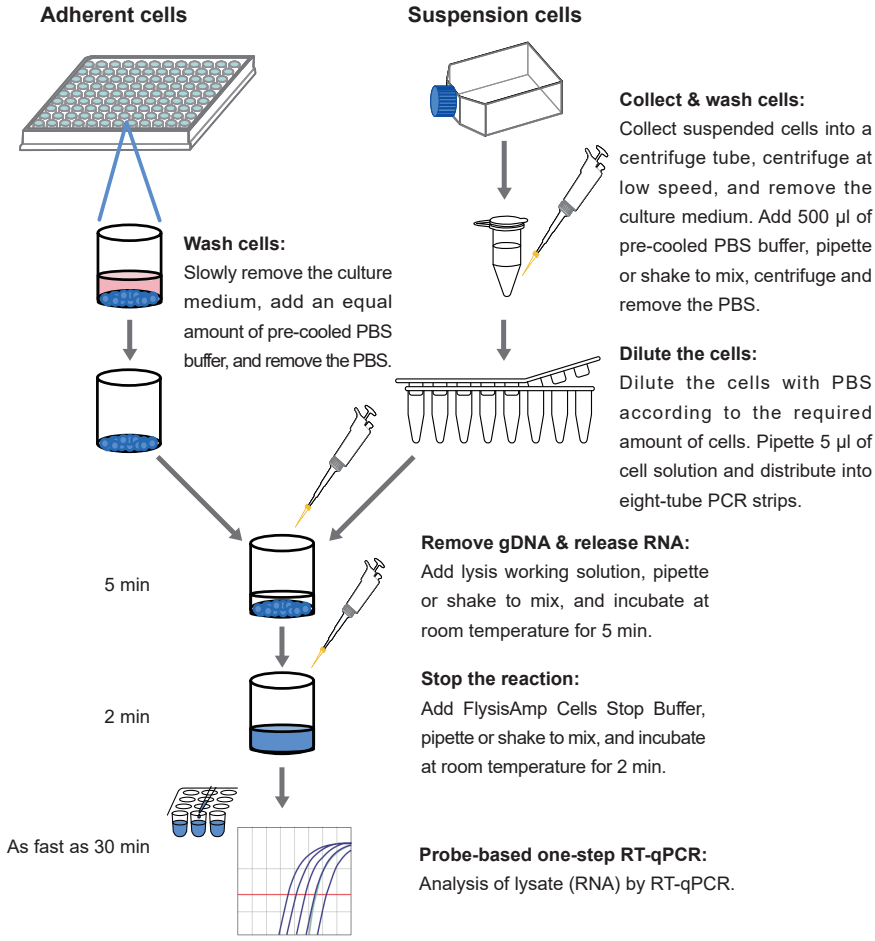

Fig 1. Workflow of FlysisAmp Cells-to-CT 1-Step Probe Kit

08/Experiment Process

08-1/RNA Acquisition

◇Adherent cells:

1. Sample processing and preparation

Gently remove the cell culture medium from the adherent cells in different well plates, then slowly add an equal volume of pre-cooled 1 × PBS to the plate to wash the adherent cells. Remove the PBS carefully, and place the cells on ice for later use.

▲ When removing culture medium or PBS, the action should be as gentle as possible to avoid cell loss during the washing process.

2. RNA acquisition

a. Prepare lysis working solution (take 96-well plate as an example)

Components	Volume	
FlysisAmp Cells Lysis Buffer	46 μl	□
DNase I	2 μl	■
Enhancer Solution	2 μl	■
Total	50 μl	

▲ Lysis working solution should be prepared according to the required volume of the well plate, the ratio of each component is FlysisAmp Cells Lysis Buffer : DNase I : Enhancer Solution = 23 : 1 : 1.

▲ After preparation, mix by inverting 10 - 15 times, avoid violent vortexing; store on ice after mixing, please use within 1 h.

b. Refer to the table below and add the corresponding lysis working solution to the aliquoted cell sample, pipette gently 8 - 10 times to mix thoroughly, and let stand at room temperature for 5 min to lyse the cells.

Components	Volume					
Cell culture plate	384 well	96 well	48 well	24 well	12 well	6 well
Lysis working solution volume	25 μl	50 μl	100 μl	150 μl	250 μl	750 μl

c. Add FlysisAmp Cells Stop Buffer as shown in the table, pipette gently 8 - 10 times to mix, and let stand at room temperature for 2 min to terminate the reaction.

Components	Volume						
Cell culture plate	384 well	96 well	48 well	24 well	12 well	6 well	
FlysisAmp Cells Stop Buffer	2.5 μl	5 μl	10 μl	15 μl	25 μl	75 μl	■
Total	27.5 μl	55 μl	110 μl	165 μl	275 μl	825 μl	

▲ The lysate can be used for subsequent detection experiments, such as reverse transcription, one-step RT-qPCR/RT-PCR.

▲ The lysate can be stored on ice for 2 h. If long-term storage is required, it should be stored at -80°C.

◇Suspension cells:

1. Sample processing and preparation

a. Remove the culture medium: Transfer the suspension cells to a centrifuge tube, centrifuge at 4°C, 1,000 rpm (930 × g) for 5 min, collect to the bottom of the tube, and remove the culture medium.

b. Wash cells: Add pre-cooled 1 × PBS (500 μl/10^5 cells), pipette gently 8 - 10 times until there are no obvious cell clusters; centrifuge at 1,000 rpm (930 × g) at 4°C for 5 min. Collect to the bottom of the tube and remove PBS.

c. Dilute cells: According to the experimental requirements, dilute the cells with pre-cooled 1 × PBS, distribute the diluted cell suspension into eight-tube strips, 5 μl per tube (keep the number of cells at 10 - 10^5 cells/tube), and place on ice.

▲ When removing culture medium or PBS, the action should be as gentle as possible to avoid cell loss during the washing process.

2. RNA acquisition

a. Preparation of lysis working solution

Components	Volume			
Cell number	10 - 10^3	10^3 - 10^5	10^5 - 10^6	
FlysisAmp Cells Lysis Buffer	24 μl	46 μl	184 μl	☐
DNase I	1 μl	2 μl	8 μl	■
Enhancer Solution	-	2 μl	8 μl	■
Total	25 μl	50 μl	200 μl	

▲ After preparation, mix by inverting 10 - 15 times, avoid violent vortexing; store on ice after mixing, please use within 1 h.

b. Refer to the table below and add the corresponding lysis working solution to the aliquoted cell sample, pipette gently 8 - 10 times to mix thoroughly, and let stand at room temperature for 5 min to lyse the cells.

Components	Volume		
Cell number	10 - 10^3	10^3 - 10^5	10^5 - 10^6
Lysis working solution	25 μl	50 μl	200 μl
Total	30 μl	55 μl	205 μl

c. Add FlysisAmp Cells Stop Buffer as shown in the table, pipette gently 8 - 10 times to mix, and let stand at room temperature for 2 min to terminate the reaction.

Components	Volume			
Cell number	10 - 10^3	10^3 - 10^5	10^5 - 10^6	
FlysisAmp Cells Stop Buffer	2.5 μl	5 μl	20 μl	■
Total	32.5 μl	60 μl	225 μl	

▲ The lysate can be used for subsequent detection experiments, such as reverse transcription, one-step RT-qPCR/RT-PCR.

▲ The lysate can be stored on ice for 2 h. If long-term storage is required, it should be stored at -80°C.

08-2/One-Step RT-qPCR

1. Prepare one-step RT-qPCR system

Components	Volume	
5 × 1-Step RT-qPCR Mix (ROX)	4 μl	☐
Primer Forward (10 μM)	0.4 μl	
Primer Reverse (10 μM)	0.4 μl	
TaqMan Probe (10 μM)	0.2 μl	
Lysate	x μl	
Nuclease-free ddH_2O	to 20 μl	

The volume of each component in the reaction system can be adjusted according to the following principles:

▲ Generally, a good result can be obtained when the final concentration of primer in the reaction system is 0.2 μM. If the result is not as expected, the primer concentration can be adjusted between 0.1 - 1.0 μM.

▲ If white precipitate is found in the Master Mix after thawing, please place it at room temperature for a short while and invert the tube upside down several times to dissolve the precipitate before use.

▲ The recommended volume of lysate is 2 - 5 μl/rxn.

No RT Control Reaction (Optional)

No RT Control Reaction is a negative control which contains no Reverse Transcriptase and is used to indicate whether there is residual genomic DNA in RNA template. Replace the 5 × 1-Step RT-qPCR Mix with 2 × All-Powerful qPCR PreMix, and the other operations remain unchanged.

Mix the following components in the qPCR tube:

Components	Volume
2 × All-Powerful qPCR PreMix	10 μl
Primer Forward (10 μM)	0.4 μl
Primer Reverse (10 μM)	0.4 μl
TaqMan Probe (10 μM)	0.2 μl
Lysate	x μl
Nuclease-free ddH_2O	to 20 μl

2. Run the RT-qPCR reaction according to the following conditions

Standard Program

Stage 1	Reverse Transcription	Rep: 1	55°C	15 min	1.6°C/sec
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec	1.6°C/sec
Stage 3	Cycles	Reps: 40	95°C	10 sec	1.6°C/sec
			60°C	30 sec	1.6°C/sec

Fast Program

Stage 1	Reverse Transcription	Rep: 1	55°C	2 min	3.19°C/sec
Stage 2	Initial Denaturation	Rep: 1	95°C	2 sec	3.19°C/sec
Stage 3	Cycles	Reps: 40	95°C	1 sec	3.19°C/sec
			60°C	10 sec	2.45°C/sec

▲ Please conduct preliminary experiments for the first attempt to confirm whether the fast program is compatible with the qPCR instrument.

▲ The "Fast" mode can be selected under "Run mode" in the "Experiment Properties" tab.

▲ Please adjust the reaction time and temperature of each step according to the actual used Real-Time PCR instrument and self requirement.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
No PCR product or low yield	1. Insufficient cell lysis	1. Insufficient addition of Lysis Buffer: Follow the recommended number of cells and well plates in the instruction. 2. Uneven mixing of cells and Lysis Buffer: When adding Lysis Buffer, extend the pipette tip below the liquid level. Mix by pipetting up and down 8 - 10 times.
	2. Stop Buffer is not added or mixed evenly	When adding Stop Buffer, extend the pipette tip below the liquid level. Mix by pipetting up and down 8 - 10 times.
	3. Insufficient cell input or cell lysate input	Increase the amount of cell input or increase the amount of lysate input according to the instructions.
	4. Excessive cell input: Excessive cell contents inhibit amplification.	Perform the experiment according to the instruction.
	5. The sample does not contain the target RNA or the expression level of target RNA is low	Increase the amount of cell input or adjust the amplification system.
	6. The frozen cells were rinsed with PBS	After freezing, the cells have broken. PBS washing results in RNA loss. It is recommended to re-culture the cells and repeat the experiment.
RNA degradation	1. RNA was degraded before the experiment	Place fresh cells on ice promptly after processing to avoid prolonged placement at room temperature.
	2. The lysate was left at room temperature for too long	The lysate should not be allowed to stand at room temperature for more than 20 min or on ice for more than 2 h. Follow-up tests should be carried out in a timely manner or the lysate should be frozen at -80°C.
	3. Excessive cell input: Cellular RNases degrade RNA	Reduce the amount of cell input and perform the experiment according to the instruction.
Genomic DNA residues	1. Excessive cell input	Cell input should be $<10^6$.
	2. Insufficient cell lysis	Extend the lysis time appropriately, but not more than 10 min.

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