LAMDA BIOTECH

PRODUCT INFORMATION

Catalog No.: **G915-1000**

Product Name: **Direct RT-qPCR Lysis Kit**

Size: **1000 Preps**

Description: **Direct RT-qPCR Lysis Kit** offers a simple and quick method to prepare template directly from 10-10^5 cultured cells. The kit includes reagents for cell lysis and gDNA removal at the same time. Without further RNA extraction and purification, the prepared template in the lysate can be used directly for reverse transcription and qPCR (two step RT-PCR); or directly for qRT-PCR (one step RT-PCR).

Kit Contents:	Components	Volume
	Lysis Solution	50 ml
	Stop Solution	6 ml
	Protease	1 ml
	Protease Inhibitor	1 ml

Note: *Try to avoid frequent Free-n-Thaw cycles. Aliquot reagents for small sample size experiments.*

Storage: Store all components at –20°C in a non-defrosting freezer.

Protocol:

1. Thaw and leave the Lysis Solution and Stop Solution at room temperature and mix gently but thoroughly.
2. Prepare 10-10^5 cells per reaction for lysis.

    - **For adherent cells grown in 96- or 384-well plates:** Aspirate the culture medium from the wells and rinse with 50 µl of chilled 1X PBS. Remove the PBS.
    - **For suspension cells:** Pellet up to 10^5 cells in a centrifuge tube. Remove the culture medium and rinse with 50 µl of chilled 1X PBS. Remove the PBS.

3. Prepare the Lysis Mix: use a new centrifuge tube, mix 50 µl Lysis Solution with 1 µl Protease for each sample. For example, if you have 5 samples, you need to mix 250 µl Lysis Solution with 5 µl Protease.
4. Prepare the Stop Mix: use a centrifuge tube, mix 5 µl Stop Solution with 1 µl Protease Inhibitor for each sample; e.g., if you have 5 samples, you need to mix 25 µl Stop Solution with 5 µl Protease Inhibitor.
5. To lyze the cells, add 50 µl of the Lysis Mix from Step 3 to the cells prepared from Step2. Mix the cells well by pipetting 35 µl of the mixture up and down for five times. Avoid creating bubbles.
6. Incubate for 10 minutes at 37°C.
7. To terminate the lysis, add 5 µl of the Stop Mix prepared at Step 4; and mix the lysate by pipetting 35 µl of the mixture up and down 5 times.
8. Incubate the final mixture for 5 minutes at room temperature. The lysates containing RNA templates is now ready for various downstream applications.

Note:

1. To minimize RNA degradation, keep the cells in PBS on ice before starting the cell lysis procedure.
2. Lysis Solution and Stop Solution must be at room temperature and mixed well before adding to the cells.
3. To protect the RNA templates in solution, we recommend using the lysates immediately within one hour. However, the lysates can be stored at -80°C for short term storage with one freeze-thaw cycle.
4. The prepared lysate should be used less than 10% of the final volume for any downstream applications.