Protocol for cell collection with NADMED lysis buffer

version 2.0

Purpose of the procedure:

Quenching of cell metabolism on the plate without enzymatic treatment. This treatment will scavenge NAD+, NADH, NADP+, NADPH, GSSG, and GSH for the NADMED assays.

Steps:

1. Warm the **LYSIS BUFFER** to 55°–60°C by incubating for 10 minutes in a water bath.

2. Wash cells with PBS to remove media. Proteins from the media will interfere with the normalization of the results. PBS should be either at room temperature or at the same temperature as culture media.

3. Remove PBS and add pre-warmed LYSIS BUFFER*. Distribute the LYSIS BUFFER volume over the cells by tilting of the culture bottle or dish. This treatment kills and partially lyses the cells.

   NOTE: Aim for concentrated LYSIS BUFFER-Cell mixture. However, the whole surface needs to be covered with the buffer for convenient harvesting of the cells.

   NOTE: If you are using 6-well plates, for example, collect cells from one well at a time to avoid differences in handling time.

   *Examples of LYSIS BUFFER volume per culture dish type:

CULTURE DISH	VOLUME OF LYSIS BUFFER
T75 flask	2 mL
10 cm culture dish	1 mL
6-well plate	400 µL (per well)

4. Collect the cells by scraping and transferring all material into a clean microtube. You should see fluffy white flakes and threads in the homogenate. These are desaturated proteins.

   NOTE: The collected volume of the homogenate will be lower than the added LYSIS BUFFER. This is expected as it has the property to evaporate.

5. Place the homogenate on ice for the time needed to collect cells from several dishes.

6. Freeze the homogenates at -80°C. Ship the samples on dry ice.