Protocol For GentleLys - Native Cell Lysis Buffer - Dissolve

Overview

The Cube Biotech GentleLys Buffer is the only 100% detergent-free, non-denaturing solution to lyse cultured mammalian cells from plated cells as well as cells pelleted from suspension cultures while solubilizing all membranes in the process. GentleLys enables the extraction of soluble proteins and the stabilization of membrane proteins from all compartments of mammalian cells. It is compatible with many applications, including Immunoassays, Protein Assays, Mass Spectrometry, and Protein Purification.
The Buffer comes in two variants: GentleLys – **Dissolve** which lyses mammalian cells in 15 min while GentleLys – **Stabilize** lyses all mammalian cells and makes the whole proteome (soluble and membrane proteins) of the cell available for your analytic approaches. It solubilizes all membranes and stabilizes expressed membrane proteins in their native conformation as well as native lipid environment in polymer-supported native nanodiscs. This process only takes two hours.

Please contact us if you have questions or need assistance optimizing a protocol for your application.
contact@cube-biotech.com

Equipment

- Ice bath
- Micropipettor
- Micropipetting tips
- Centrifuge
- Cellscraper
- pH meter
- Vortex mixer
- End-over-end shaker
- 2 ml Eppi Tubes

Materials

- Cell Suspension Culture
- 1 vial of GentleLys - Native Cell Lysis Buffer - Dissolve
- Add 1 ml ddH_2O to dissolve the lyophilized Buffer to restore:
  - 20mM HEPES pH 7.5
  - 100 mM NaCl
  - Special Engineered Copolymer

Guidelines

▪ GentleLys Buffers do not contain protease or phosphatase inhibitors. If desired, add protease inhibitors to the Buffer prior to the assay.

▪ Use 1ml of cold GentleLys Buffer for every 5×10^6 of cells (20 µl of packed cells, which is equivalent to 40 mg of cells).
GentleLys can be used to lyse cells on plates to obtain concentrated protein extracts with the use of less Buffer.

▪ Since GentleLys is detergent-free and non-denaturing it does not interfere with the activity of soluble proteins as well as protein kinases and other enzymes/ membrane proteins.

▪ All GentleLys Buffers are compatible with protein concentration determination assays like BCA. The Buffers do not absorb at 280nm.

▪ GentleLys Buffers come as lyophilized powder to ensure stability and increased shelf life. Prior to use just add 1 ml ddH_2O and mix until fully dissolved.
Dissolved GentleLys Buffer can be stored away from light at 4°C for up to 4 weeks.

Lyse Monolayer-cultured mammalian cells with GentleLys - Dissolve

Note: If desired, add protease and phosphatase inhibitors to the GentleLys Buffers immediately before use. Remember that protease inhibitors can change the pH of GentleLys Buffers.

1. Carefully remove the culture medium from the adherent cells.
2. Wash cells twice with a cold Buffer of choice.
3. Add cold GentleLys - Dissolve Buffer to the cells. Use 1 ml of Buffer per 75 cm^2 flask containing 5 × 10^6 cells. Keep on ice for 5-15 minutes, swirling the plate occasionally for uniform spreading.
4. Collect the lysate at one side of the flask using a cell scraper and transfer it to a microcentrifuge tube. Centrifuge samples at 14,000 × g for 5 minutes to collect the cell debris.
5. Transfer the supernatant to a new tube for further analysis.

Note

Lower centrifugation speeds might need longer centrifugation time. Adapt accordingly.

Lyse Suspension-cultured mammalian cells with GentleLys - Dissolve

Note: If desired, add protease and phosphatase inhibitors to the GentleLys Buffers immediately before use. Remember that protease inhibitors can change the pH of GentleLys Buffers.

1. Collect cells by centrifugation at 2500 × g for 5 minutes. Discard the supernatant.
2. Wash cells twice in a cold Buffer of choice. Collect cells by centrifugation at 2500 × g for 5 minutes.
3. Add GentleLys Buffer to the cell pellet. Use 1 ml of GentleLys Buffer for 40 mg (5 × 10^6 of cells) of wet cell pellet. Resuspend the pellet by pipetting the mixture up and down.
4. Shake mixture gently for 15 minutes at 4 °C. Centrifuge mixture at 14,000 × g for 5 minutes to pellet the cell debris.
5. Transfer the supernatant to a new tube for further analysis.

Note

Quantities can be scaled up accordingly.

Note

Lower centrifugation speeds might need longer centrifugation time. Adapt accordingly.