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Product No. 22353

Cell Lysis Buffer (10X)

Features

- Among the most reliable buffers used for lysing cultured mammalian cells while preventing proteolysis and interference with immunoreactions and biological activity.
- Contains Protease Inhibitor Cocktail. It is not necessary to add protease inhibitors (e.g., PMSF).
- SDS solution is provided with Cell Lysis Buffer but is not premixed. Suitable for immunoprecipitation, where SDS may adversely affect the antigen–antibody reaction.
- The preservative used in Cell Lysis Buffer does not affect the antigen–antibody reaction or protein extraction.

Components

Reagents	Volume	Quantity	Bottle
Cell Lysis Buffer with Protease Inhibitor Cocktail but without SDS (10X)	2 mL	5	Umber tube
SDS solution (1% SDS)	2 mL	5	Clear tube

Required reagents

Water deionized and sterilized (Product No.06442) or ultrapure water (protease and protein free).

Composition

1X Solution

50 mmol/L Tris-HCl buffer (pH 7.6), 150 mmol/L NaCl, 1%(w/v) CHAPS, 0.5%(w/v) sodium deoxycholate, Protease Inhibitor Cocktail (1X), preservative (0.1%(w/v) SDS, preservative)

Preparation

1. Thaw Cell Lysis Buffer (10X) and SDS solution completely at room temperature before vortexing.
2. Mix 800 µL of water, 100 µL of Cell Lysis Buffer (10X), and 100 µL of SDS solution in a microtube.

- For different volumes, use the mixture ratio 8:1:1 for water : Cell Lysis Buffer (10X) : SDS solution.
- To prepare Cell Lysis Buffer without SDS, use the mixture ratio 9:1 for water : Cell Lysis Buffer (10X).
- Store 1X Cell Lysis Buffer at −20°C. The use of additional protease inhibitors [e.g., Protease Inhibitor Cocktail (EDTA free) (Product No.03969)] is recommended when 1X Cell Lysis Buffer has been stored for over one month.

Protocol

A) For suspension cells

1. Remove the medium from cultured cells and wash them twice with cold D-PBS (−).
2. Remove D-PBS (−), add 1X Cell Lysis Buffer to the cell pellet, and vortex (add 1X Cell Lysis Buffer at 0.5–5.0 × 10^7 cells/1 mL Cell Lysis Buffer).
3. Fragment the DNA by passing the lysed suspension through a needle (21 gauge) attached to a syringe (this procedure can be skipped, but the protein yield may be increased through DNA fragmentation).
4. Incubate the samples for 15 min on ice (to increase the yield, extend the incubation period).
5. Centrifuge at 10,000 ×*g* and 4°C for 10 min.
6. Transfer the supernatant containing the total protein extracts to a new tube for further analysis.

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B) For adherent cells

1. Remove the medium from cultured cells and wash them twice with cold D-PBS (−).
2. Add 1X Cell Lysis Buffer to the culture dish and stir slowly for 5 min
   (add 1X Cell Lysis Buffer at 0.5–5.0 × 10^7 cells/1 mL Cell Lysis Buffer).
3. Fully scrape the cells using a cell scraper.
4. Transfer the lysate with the pellet to a new tube.
5. Wash the culture dish with 400 µL of 1X Cell Lysis Buffer and pool the solution in a collection tube.
6. Fragment the DNA by passing the lysed suspension through a needle (21 gauge) attached to a syringe
   (this step can be skipped, but the protein yield may be increased through DNA fragmentation).
7. Incubate the samples for 15 min on ice (to increase the yield, extend the incubation period).
8. Centrifuge at 10,000 ×*g* and 4°C for 10 min.
9. Transfer the supernatant containing the total protein extracts to a new tube for further analysis.

C) For tissue

1. Chop tissue into pieces using a scalpel.
2. Add 3 mL of 1X Cell Lysis Buffer to 1 g of tissue on ice.
3. Homogenize the tissue cells on ice.
4. Incubate the samples for 0.5–1.0 h on ice.
5. Centrifuge at 10,000 ×*g* and 4°C for 10 min.
6. Transfer the supernatant containing the total protein extracts to a new tube for further analysis.

Attention

- Add Phosphatase Inhibitor Cocktail (Product No.07574) or Phosphatase Inhibitor Cocktail (EDTA free) (Product No.07575) to Cell Lysis Buffer when conducting phosphoprotein research.
- If highly viscous substances appear during protein extraction, either increase the amount of Cell Lysis Buffer or pass the lysed suspension through a needle (21 gauge) attached to a syringe 5–10 times.

Storage

Storage temperature is stated on the product label.

Expiration

Expiration date is stated on the product label.

Packing

1 SET (for 100 mL 1X Cell Lysis Buffer) (Product No. 22353-81)

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