PROMPT
Proteins for Research

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IP Lysis Buffer

G2038

Introduction

IP Lysis Buffer is a lysate that lysates cells or tissues to prepare protein samples under non-denaturing conditions. Protein samples obtained from tissue or cell lysis with this lysate can be applied to PAGE, western blot and immunoprecipitation (IP), co-immunoprecipitation (co-IP), Chromatin Immunopre-cipitation (ChIP) and ELISA where protein activity is required. This product consists of 25mM Tris-HCl, 150mM NaCl, 1mM EDTA and 1% NP-40 can be applied to animal or plant tissue and cell samples, as well as for fungal or bacterial samples.

Size

1×100mL

Form

Colorlessliquid

Storage

Ship with wet ice; Store at 2-8℃ away from light; Valid for 12 months.

Protocol

Requires self-provide protease inhibitors. Protease inhibitors should be added to IP lysate before use to prevent protein degradation. All references to IP lysates in the following instructions have been added with protease inhibitors.

- **For tissue samples:**
  1. The tissue blocks are washed with pre-cooled PBS to remove blood stains, and then cut into small pieces and placed in the homogenizer.
  2. Add 10 times tissue volume of IP Lysis Buffer and homogenize at low temperature. Note that the amount of IP Lysis Buffer can be added at a ratio of approximately 50 mg of tissue to 1mL of lysate. If the tissue protein content is low, the amount of lysate can be reduced to increase the protein concentration in the crude extract solution.
  3. Transfer the homogenate to a 1.5mL of centrifuge tube and shake. Ice bath for 30 minute with repeated pipetting every 10 min to ensure complete lysis of tissue cells;
  4. Centrifuge at 12000 x g for 5 minute and collect the supernatant, as total protein solution.

- **For adherent cells:**
  1. Wash the cells with PBS 2-3 times, and aspirate the residual liquid thoroughly at the last time.
  2. Absorb IP Lysis Buffer into the cell culture plate and flask at the ratio of 250μL of lysate per well of the 6-well plate, shake the culture plate and flask repeatedly to make the lysate fully contact with the cells for 3-5 minute.
  3. Scrape off the cells with a cell scraper and collect them into a centrifuge tube.
  4. Centrifuge at 12000 x g for 5 min and collect the supernatant as total protein solution.
- **For suspension cell samples:**
  1. Cells were collected by centrifugation.
  2. Mix the cytosol with IP lysate at a ratio of 250μL of lysate per cell well of a 6-well plateand shake.
  3. Ice bath for 30 minute, repeatedly pipetting every 10 minute to ensure complete cell lysis.
  4. Centrifuge at 12000 x g for 5 minute and collect the supernatant as total protein solution.
- **For bacterial or fungal samples:**
  1. 1mL of the bacterial suspension was centrifuged to remove the supernatant and washed once with PBS to fully remove the liquid. The vortices make the thalli disperse as much as possible.
  2. Add 100-200μL of IP lysate and vortex gently to mix well with the lysate.
  3. Ice bath for 10 minute, repeatedly pipetting every 2 minute to ensure complete lysis of thalli.
  4. Centrifuge at 12000 x g for 5 min and collect the supernatant as total protein solution.

Note

1. Tissue or cells may appear viscous when lysed. This can be achieved by repeatedly blowing with a pipettor or shaking with a vortexer until it becomes a liquid. Add a further appropriate amount of lysis solution if it remains thick.
2. This reagent does not contain protease inhibitors;add your own protease inhibitors before use.
3. The total protein solution obtained by cleavage of this product.
4. For your safety and health, please wear safety glasses, gloves, or protective clothing.