RIPA Total Protein Extration Lysis Buffer(strong)

Introduction:

RIPA total protein extration lysis buffer is a traditional fast lysate of cell tissue. RIPA Protein samples obtained from lysis solution can be used for conventional Western, IP and ELISA. RIPA means Radio Immunoprecipitation Assay. RIPA There are many kinds of formulas of pyrolysis liquor. According to the strength of pyrolysis liquor, it can be roughly divided into three categories: strong, medium and weak. Because the product contains a high concentration of detergent, the protein concentration of the sample obtained from the pyrolysis solution can not be determined by Bradford method.

Contents:

Cat No: BD0032

RIPA Lysis Buffer 100ml

Procedure:

For cultured cell samples:

1. Melt RIPA lysis and mix. The final concentration of PMSF is 1 mM by adding PMSF in a few minutes before the use of appropriate amount of pyrolysis solution.

2. For Adherent cells：Remove the inoculum and wash it once with PBS, saline or serum-free culture medium (if there is no interference with the protein in the serum, it can not be washed). Add the lysate according the ratio of 6 hole plates added to each hole with 150-250ul lysis. Blow it down with the gun, made lysis fully contacted with cells. Usually the cells will be splitting after 1-2s.

For suspension cells: Centrifugally collect cells and use fingers to force cells to disperse. Add the lysis according the ratio of 6 hole plates added to each hole with 150-250ul lysis. Use fingers to flick the cells. There should be no obvious cell precipitation after full lysis. If there are more cells, it must be packed into 50-100 thousands of cells / tubes before splitting.

3. After full pyrolysis, centrifugation of 10000-14000g for 3-5 minutes and supernatant removal, subsequent PAGE, Western and immunoprecipitation operations can be carried out.

The amount of lysis: usually 150 ul lysis is enough for each cell of 6-well plate is enough, but if the cell density was very high, the amount of pyrolysate could be increased to 200 microliters or 250 microliters. The protein concentration of supernatant obtained from 100ul of this product is about 2-4 mg/ml per million cells, which differs from cell to cell.

For tissue samples：

1. Cut the tissue into tiny pieces.

2. Melt RIPA lysis and mix well. The final concentration of PMSF is 1 mM by adding PMSF in a few minutes before the use of appropriate amount of pyrolysis solution.

3. Add the buffer according the ratio of every 20㎎ with 150-250ul lysis. (If the cracking is not enough, can add more lysis，If a high concentration of protein is needed, can reduce the amount of lysis).

4. Homogenate with glass homogenizer until full lysis.

5. After full pyrolysis, centrifugation of 10000-14000g for 3-5 minutes and supernatant removal, subsequent PAGE, Western and immunoprecipitation operations can be carried out. The supernatant obtained from each 20 mg cryopreserved mouse liver was lysed with 200ul of lysate, and the protein

concentration was about 15-25 mg/ml. Different tissues in different states were different.

6. Explanation: If the tissue sample itself is very small, it can be sheared properly and cracked directly with pyrolysis solution. The sample can be fully cracked by strong vortex. Then the supernatant was centrifuged for subsequent experiments. The advantage of direct pyrolysis is that it is more convenient and does not need to use homogenizer. The disadvantage of direct pyrolysis is that it is more sufficient than that of homogenizer.

Note: A small group of transparent colloidal substances often appears in the pyrolysis products of RIPA lysis, which is a normal phenomenon. The transparent gelatin is a complex containing genomic DNA, etc. The supernatant can be directly centrifuged for subsequent experiments without detecting proteins that bind closely to genomic DNA. If it is necessary to detect proteins that bind tightly to genomic DNA, the transparent gelatin can be broken and dispersed by ultrasound treatment, and then centrifuged for subsequent experiments.

Attention:

In order to achieve the best use effect, try to avoid excessive repeated freezing and thawing. It can be properly packed and used.

PMSF need to self provide.

All steps should be carried out on ice or at 4℃.

The product is limited to the scientific research of professionals. It can not be used for clinical diagnosis or treatment. It can not be used for food or medicine. It can not be stored in ordinary houses.

For your safety and health, please wear test clothes and disposable gloves.

Storage & Shelf life:

Store at -20℃ , Period of validity for one years .

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