

## Product Data Sheet

Product Name: RIPA Lysis Buffer (Medium)

Cat. No.: GK10024

### Components

| Catalog No.                                         | Components                 | 100 mL | Storage |
|-----------------------------------------------------|----------------------------|--------|---------|
| GK10024-R                                           | RIPA Lysis Buffer (Medium) | 100 mL | -20°C   |
| GK10024-P                                           | PMSF (100mM)               | 1 mL   | -20°C   |
| Stored at -20°C, and is stable for up to 12 months. |                            |        |         |

### Protocol

#### 1. Cell sample:

(1) Melt RIPA Lysis Buffer and mix well. Take an appropriate amount of lysate and add PMSF before use to make the final concentration of PMSF 1mM. Appropriate Cocktails of the above-mentioned protease phosphatase inhibitors can be added according to experimental needs.

(a) Adherent cells: discard the culture medium and wash it with PBS, normal saline or serum-free culture medium (if there is no interference with the protein in the serum, it is not necessary to wash it). Add 150-250 uL lysate to each well of the 6-well plate. Use a gun to blow for several times to make the lysate fully contact the cells (in general, the cells will be lysed after the lysate contacts the animal cells for 1-2s). Plant cells should be lysed on ice for 2-10 min.

(b) Suspended cells: Collect cells by centrifugation, gently vortex or flick the bottom of the tube to disperse the cells as much as possible. Add the lysate at the ratio of 150-250 microliters of lysate to each well of the 6-well plate. Flick the bottom of the tube to fully lyse the cells. There should be no obvious cell sedimentation after full lysis. If the amount of cells is large, it must be divided into 500,000-1,000,000 cells/tube, and then lysed. Large clusters of cells are more difficult to lyse sufficiently, while a small amount of cells are relatively easy to lyse sufficiently because the lysis solution is easy to fully contact with the cells.

(c) Bacteria or yeast: Take 1 mL of bacterial liquid or yeast liquid, centrifuge to remove the supernatant (or use PBS to wash once to fully remove the liquid), gently vortex or flick the bottom of the tube to disperse the bacteria or yeast as much as possible. Add 100-200 uL lysis buffer, gently

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vortex or flick the bottom of the tube to mix, and lyse on ice for 2-10 minutes (If you want better lysis effect, bacteria and yeast can use lysozyme and breaking enzyme (Lyticase) digestion, and then use this lysis solution for lysis).

(2) After full lysis, centrifuge at 10000-14000g for 3-5 min, take the supernatant, and then perform the subsequent PAGE, WB and IP operations.

### 2. Tissue samples:

(1) Cut the tissue into small pieces.

(2) Melt RIPA Lysis Buffer (Medium) and mix well. Take an appropriate amount of lysate and add PMSF before use to make the final concentration of PMSF 1mM. Appropriate Cocktails of the above-mentioned protease phosphatase inhibitors can be added according to experimental needs.

(3) Add lysate at the ratio of 150-250 uL lysate per 20 mg of tissue. (If the lysis is insufficient, you can appropriately increase the amount of lysate. If you need a high-concentration protein sample, you can appropriately reduce the amount of lysate.)

(4) Homogenize with a glass homogenizer until it is fully lysed. Or freeze the tissue sample and grind it with liquid nitrogen, and add the lysis solution for lysis after the grinding is complete.

(5) After full lysis, centrifuge at 10000-14000g for 3-5min, take the supernatant, and then perform the subsequent PAGE, WB and IP operations.

### Precautions

(1) Instructions for the amount of lysis solution: usually the amount of bacteria and yeast in each well of a 6-well plate or 1 mL of bacteria or yeast is enough to add 150 uL of the lysis solution. If the cell density is very high, you can increase the amount of the lysis solution. The dosage is 200 uL or 250 uL. The supernatant obtained by lysing 100 uL of this product for every 1 million animal cells has a protein concentration of about 2-4 mg/mL, which varies from cell to cell.

(2) The supernatant obtained after every 20mg of frozen mouse liver tissue is lysed with 200 uL of this lysate, the protein concentration is about 15-25mg/mL, and different tissues in different states are different.

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(3) If the tissue sample itself is very small, it can be directly added to the lysis solution after appropriate shearing, and the sample can be fully lysed by vigorously vortexing. Then the supernatant was collected by centrifugation and used in subsequent experiments. The advantage of direct pyrolysis is that it is more convenient and does not need to use a homogenizer or grinding equipment. The disadvantage is that it is not as adequate as homogenization or grinding.

(4) A small group of transparent jelly often appears in the lysate of RIPA lysate. The transparent jelly is a complex containing genomic DNA, which is a normal phenomenon. In the case of not detecting proteins that are tightly bound to genomic DNA, the supernatant can be directly centrifuged to obtain the supernatant for subsequent experiments; if you need to detect proteins that are tightly bound to the genome, the transparent gel can be broken up by ultrasonic treatment, and then Centrifuge to take the supernatant for subsequent experiments. If some common transcription factors are detected, such as NF-kappaB, p53, etc., these transcription factors can usually be detected without ultrasonic treatment.

(5) In order to obtain the best use effect, it is recommended to avoid repeated freezing and thawing as much as possible after proper packaging.

(6) All steps of lysing samples need to be performed on ice or 4°C.

### Background

RIPA Lysis Buffer (Medium) (RIPA Lysis Buffer (Middle)) is a commonly used rapid lysis solution for cell tissues. Cells or tissue samples are lysed under non-denaturing conditions to prepare protein samples. The main ingredients of this product are 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40 (ethyl phenyl polyethylene glycol), 0.5% sodium deoxycholate (sodium deoxycholate), 0.1% SDS, and Sodium orthovanadate (sodium orthovanadate), sodium fluoride (sodium chlorite), leupeptin (leupeptin) and other inhibitors can effectively inhibit the degradation of proteins and maintain the interaction between the original proteins. The protein sample lysed with RIPA Lysis Buffer (Medium) can be used for conventional PAGE, Western blotting (WB), immunoprecipitation (IP), etc. This product can be used for animal and plant cell or tissue samples, as well as fungal or bacterial samples.

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