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Animal Tissue/Cell Total Protein Extraction Kit

Cat: BC3790

Specification: 50T

Validity Period: RT, 1 year

Product Description:

This kit can be used for total protein sample preparation of animal cell and tissue, which is a new instrument of extracting protein quickly, suitable for SDS-PAGE and WB. Commen RIPA buffer will reduce the effective of protein extraction. This product can extract total protein more quickly and effectively with the special lysis solution, which make the outcome of WB experiment more accurate. It is only used for scientific research. The minimum volumn of the sample is 20μL.

Note:

1. protease inhibitor is not necessary but we suggest to add if the experiment takes long time or the extracted protein need to be kept for long time. The BCA kit is recommended for protein concentration determination. Lysis solution should be added to phosphatase inhibitors before use during protein phosphorylation experiment.
2. Mixed extract protein with loading buffer thoroughly before adding sample in WB.

Product Content:

Name	50 T
Denaturate Lysis Solution	25 mL
Centrifuge Tube Column	50 pcs
Collect Tube	50 pcs
Plastic Grinding Pestle	2 pcs

Operation Method (for reference only):

Cell sample

A. Non-adherent cell

1、 Precooling Centrifuge Tube Column and Collect Tube on the ice.

2、 Collect cell with low-speed centrifugation, add precooling PBS in 1.5ml cenreifuge tube, then vortex and centrifugate at 500g for 2-3min. The supernatant is removed and the remaining PBS of the same volume as the cell is left. Vortex oscillations resuspend cells.

3、 Add Denaturate Lysis Solution as the table 1, Lyse cells by vortexing(reference the table 1 to get the better extract effectivity). The cells which is not lysis thoroughly are not influent the sample quality.

4、 Transfer the lysis cell to the precooling combinatorial column tube, 14000-16000xg, 30s.

5、 Put the collect tube on ice at once, discard the centrifuge tube column, the extract protein can be used for next step experiment.

Table 1

Cell Volumn(μl)	Denaturate Lysis Solution (μl)	Cell Amount x10^6
3	20	0.3
5	50	0.5
10	100	1
20	200	2
40	500	3

B. Anchorage-dependent cell

1. Precooling centrifuge tube column and collect tube on the ice.
2. Add the precooled PBS to culture plate, culture dish or culture bottle directly, wash cells and suck out supernatant.
3. Add Denaturate Lysis Solution as the table 2, blow some times with transferpettor to mix thoroughly, Transfer the lysis cell to the precooling combinatorial column tube, 14000-16000xg, 30s. Reduce the volumn of lysis solution if extract concentrate is low.
4. Put the collect tube on ice at once, discard the centrifuge tube column, the extract protein can be used for next step experiment.

Table 2

Plate	Cell Amount	Denaturate Lysis Solution (μl)
24 well plate	0.1-0.2 Million	50
6 well plate	0.6-0.8 Million	200
25 cm^2 culture bottle	1.5-2 Million	500

Animal tissue

15-20 mg tissue as an example, adjusting the volum of Denaturate Lysis Solution according to the sapmle weight please.

1) Precooling centrifuge tube column and collect tube on the ice.
2) Put 15-20mg tissue in the centrifuge tube colume, grinding with plastic grinding pestle for 50-60 times add 200ul lysis solution, grinding with plastic grinding pestle for 30-60 times. Do not add tissue and lysis solution too much. Denaturate Lysis Solution can be added twice for best results.

Note: Plastic grinding pestl can be reused for many times, wash with distilled water and dry with tissue after using.

3) Cover and incubate at RT for 1-2 min, 14000-16000xg, 1-2 min, the supernatant is the denaturate total protein. The cells which is not lysis thoroughly are not influent the sample quality.

Problem and solution

Problem	Solution
The sample after lysis is too sticky to blow with 200-1000ul transferpettor.	Put the sample after lysis to centrifuge tube column or cut pointed end of the tips.
There are still lysis solution after centrifuging for 30s.	Reduce cell or tissue or increase volume of lysis solution.
Protein concentration is too low.	Increase cell or tissue or reduce volume of lysis solution.
The band of high MW protein (100-300KDa) is weak.	Increase volume of lysis solution to make lysis thoroughly.