Mammalian Protein Extraction Kit

Project number: M665813 (100 preps)
Storage condition: Protease Inhibitor Cocktail: -20℃, other components: room temperature.

Product content

individual parts making up a compound	M665813 100 preps
Mammalian Protein Extraction Reagent	100 ml
Protease Inhibitor Cocktail (100×)	1 ml

Product Introduction

This product is a rapid, gentle and efficient lyser of mammalian cells for the extraction of cytoplasmic and nuclear proteins. The mild formulation ensures that the extracted proteins remain biologically active and can be used in a variety of protein assays, such as reporter gene and enzyme activity assays, immunoassays, and protein purification. The extracted proteins can be analyzed by BCA for protein quantification. The kit contains a mixture of protease inhibitors to prevent protein degradation during the extraction process.

Caveat

1. This product can effectively lyse adherent cells cultured in cell culture plates (without scraping) and suspended cells collected by centrifugation, and has a higher extraction efficiency than repeated freeze-thawing or sonication. However, for tissue protein extraction, the use of the Tissue Protein Extraction Kit (CW0891) is recommended.
2. Table 1 shows the optimal amount of protein to be used for protein extraction from adherent cells. Collecting the cells first reduces the amount of reagents used and results in a higher protein concentration.
3. The amount of extraction reagent to be used can also be estimated from the number of cells. For example, if 2×106 Hela cells weigh about 20 mg, 200 μl of extraction reagent should be added.

4. The protein extracted by this product can be analyzed by BCA method for protein quantification.

Operation process

Protein extraction from adherent cells
1. Please take out the Mammalian Protein Extraction Reagent for pre-cooling before protein extraction.
2. Carefully decant the culture medium from the adherent cells and rinse the cells with PBS.
3. Add appropriate amount of Mammalian Protein Extraction Reagent (add Protease Inhibitor Cocktail at 1:99 ratio 2-3 minutes prior to protein extraction), blow the adherent cells with a lance tip on ice, transfer the lysate to a centrifuge tube, and incubate on ice for 20 minutes to allow the cells to fully lysed (refer to Table 1 for the amount of reagent to be used). The amount of reagents used should be referred to Table 1, and the time on ice should be adjusted according to the cell type.)
4. Centrifuge at 14,000 x g for 5-10 minutes.
5. Transfer the supernatant to a new tube for further analysis.

:: Protein extraction from suspension cells
1. Please take out the Mammalian Protein Extraction Reagent for pre-cooling before protein extraction.
2. Suspend the cells by centrifugation at 2,500 x g for 10 minutes and discard the supernatant. Rinse the cells with PBS. 2,500 x g, centrifuge for 10 minutes, discard the supernatant.
3. Add appropriate amount of Mammalian Protein Extraction Reagent, and add Protease Inhibitor Cocktail, i.e., 1× working solution, at the ratio of 1:99 for 2-3 minutes before protein extraction.
4. Add at least 1 ml of 1× working solution for every 100 mg of cells. For larger samples, resuspend the cells first with a small amount of 1× Work Solution and then add the remaining Work Solution.
5. After blowing, leave on ice for 20 minutes to allow the cells to fully lyse (the time on ice should be adjusted according to the cell type).
6. Centrifuge at 14,000 x g for 15 minutes.
7. Transfer the supernatant to a new tube for further analysis.

Table 1: Recommended volume of extraction reagents to be used

Cell culture plate type or flat dish type	Extraction reagent usage
100 mm	500-1,000 µl
60 mm	250-500 µl
6-well culture plate	200-400 µl / well
24-well culture plate	100-200 µl / well
96-well culture plate	50-100 µl / well

Table 2: Frequently asked questions and solutions

concern	Possible causes
low extraction rate	low protein expression
low extraction rate	Insufficient use of reagents
low extraction rate	Reagents cannot dissolve cell membranes
No access to membrane proteins	This product is more suitable for extracting nucleoplasmic proteins