For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

PRO-PREP™ Protein Extraction Solution

Cat. No. **17081** 100ml (minimum 200 preps.)

DESCRIPTION

By using PRO-PREP™, proteins can be simply extracted from all kinds of cells and tissues. The kit contains 5 kinds of protease inhibitors so it is possible to extract very highly purified proteins .

CONSIDERATION BEFORE USE

Usually detergent used in protein extraction consists of both hydrophobic tail as an amphiphilic molecule and hydrophilic head. The two parts are joined to form a micelle, that is, solubilized protein forming a lipid-detergent mixed micelle and transmembrane protein forming a protein-lipid-detergent complex. The extent of micelle formation is termed as CMC (critical micelle concentration), which is important for high efficiency as well as high purity of protein extraction. CMC is influenced by pH, temperature, ionic strength, multivalent ions of organic solvents, purity of detergent, and so on.

Depending on its ionic characteristics, a detergent can be categorized as ionic detergent, non-ionic detergent, and Zwitterionic detergent. Ionic detergent can be further classified into either cationic detergents : SDS, LiDS and DOC and anionic detergent. Thus these are highly denaturant which have a specific property to isolate protein as a monomeric form and so often used in Western blot analysis and measurement of molecular weight. Also non-ionic detergent such as Triton X100 are less protein denaturant and often employed in protein-protein interaction.

Zwitterionic detergent such as CHAPS have both negative and positive charge head at the same time, more effective in protein-protein interaction than non-ionic detergent and its extent of protein denaturation is less than that of ionic detergent. It is very important to select the optimal buffer and detergent when extracting proteins.

ADDED PROTEASE INHIBITORS

PMSF	- inhibits serine protease and thio protease - added in a working concentration of 1.0mM (174μg/ml)
EDTA	- inhibits metaloprotease - added in a working concentration of 1.0mM
Pepstatin A	- inhibits acid protease - added in a working concentration of 1uM (0.7μg/ml)
Leupeptin	- inhibit serine protease - added in a working concentration of 1uM (0.5μg/ml)
Aprotinin	- inhibit serine and thiol protease - added in a working concentration of 0.1uM (2.0μg/ml)

CHARACTERISTIC

1. When extracting proteins from cells or tissues, one doesn't necessarily apply appendix treatment.
2. Able to minimize protein extraction time into 20-30 minutes.
3. Protein stabilization buffer can make protein stable.
4. There is no protein degradation due to freezing or thawing since there is no freezing at -20°C reservation.
5. Extracted proteins are stable for more than 6 months when kept in –20°C.
6. There is no absorbable error because there is no absorbable hindrances of PRO-PREP solution when measuring protein concentration.
7. Very useful for protein separation in Western blot analysis because Ionic detergent turns protein into monomers.
8. Very useful for protein molecular weight analysis because denature protein into a monomers.
9. Protein degradation is minimized by adding commonly used protease inhibitor and doesn't necessarily need to prepare protease inhibitor.

PROTOCOL (For Cells)

1. Preparation of cells.

   Note : After preparation of adherent cell or suspension cell in 50ml tube, centrifuge at 2,000-3,000rpm for 5 min. Then wash cells with PBS/DPBS (optional). After washing, count cells and use approximately $5x10^6$ cells.

2. Harvest the cell pellet by centrifuge at 13,000rpm for 10-20 seconds.

   Note : After centrifugation, remove the remnant using a pipette.

3. Resuspend cells in 400μℓ PRO-PREP™ solution, and mix well.

   Note : Depending on tissue types, one can vary volume of PRO-PREP™ solution. Generally add $5x10^6$ cell per 400μℓ, but determine the optimal amount of solution according to cell size. Also, pipette carefully as the addition of PRO-PREP™ solution can produce bubbles.

4. Induce cell lysis by incubation for 10-20 min on ice or freezer at –20°C.

   Note : PRO-PREP™ solution don't freeze at -20°C, and it can be stabilize protein refraining protein degradation with protease inhibitor. Before incubating, it can also increase cell lysis using a syringe(optional). At this time, there appears bubbles, yet doesn't need to care because they disappear during centrifuging or incubation.

5. Centrifuge at 13,000rpm (4°C) for 5minutes, and transfer supernatant to a fresh 1.5ml tube.
6. Measure of protein concentration.

   Note : When measuring protein concentration by Bradford' method etc., PRO-PREP™ solution is made to have no absorbable hindrance, and so can decline an absorbable error.

PROTOCOL (For Tissues)

1. Preparation of tissue about 10-20mg.

   Note : After digging the interested tissue, transfer it to an appropriate tube. Keep the tissue fresh as much as possible.

2. Homogenize tissues in 600μℓ PRO-PREP™ solution.

   Note : According to tissues, can be have different addition of PRO-PREP™ solution. Generally add 10mg tissue per 600μℓ, but determine to add the optimal amount of solution for each experiment. Also, when tissue is homogenized by homogenizer, there appears bubbles. If incubated or centrifuged, they will disappear. Doesn't need to care.

3. Induce cell lysis by incubation for 20-30 min on ice or freezer at –20°C.

   Note : PRO-PREP™ don't freeze at -20°C, and it can be stabilize protein refraining protein degradation with protease inhibitor. Before incubating, it can also increase cell lysis using a syringe(optional). At this time, there appears bubbles, yet doesn't need to care because they disappear if centrifuged or incubated in freezer.

4. The following procedures are same as the method For Cells.