ISO 9001/14001 Certified Company

PRO-PREP™ Protein Extraction Solution

Cat. No. 17081 100 ml

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 and hydrophilic head as an amphiphilic molecule. The two parts are joined to form a micelle, that is, soluble 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 both 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 X-100 are less potent as 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 1μM (0.7μg/ml)
Leupeptin	- inhibit serine protease - added in a working concentration of 1μM (0.5μg/ml)
Aprotinin	- inhibit serine and thiol protease - added in a working concentration of 0.1μM (2.0μg/ml)

CHARACTERISTIC

1. When extracting proteins from cells or tissues, one doesn't necessarily apply any other treatments.
2. Able to minimize protein extraction time within 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 preservation.
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 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. And then transfer to the new 1.5ml tube.
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 : Generally add 400μl per $5x10^6$ cell , 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 stabilize protein by 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 centrifugation 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 adding different addition of PRO-PREP™ solution. Generally add 600μl per 10mg tissue , 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 stabilized 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 PROTOCOL of cells.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• Yield

1. Protein extraction volume in various cells and tissues.

The conclusion of total protein volume used by PRO-MEASURE™ Protein Measurement Solution after isolate protein used by PRO-PREP™ Protein Extraction Solution from various cells or tissues is average 2mg per 5 X 10^6 cells and 8-9mg per 10mg tissues.

1> Celll

Strain	Cell Number	Protein
K562 (human)	5 X 10^6	2.04 mg
SNU 601 (human)	5 X 10^6	1.89 mg
YAC 1 (mouse)	5 X 10^6	2.10 mg
B16 (mouse)	5 X 10^6	1.90 mg

2>Tissue

Strain	Cell Number	Protein
Spleen (mouse)	10 mg	9.01 mg
Kidney (mouse)	50 mg	9.91 mg
Lung (mouse)	10 mg	9.67 mg
Liver (mouse)	10 mg	6.59 mg

2. SDS-PAGE gel Electrophoresis conclusion

Total proteins were isolated from various cancer cell lines and tissues by the PRO-PREP ™ Protein Extraction Solution.

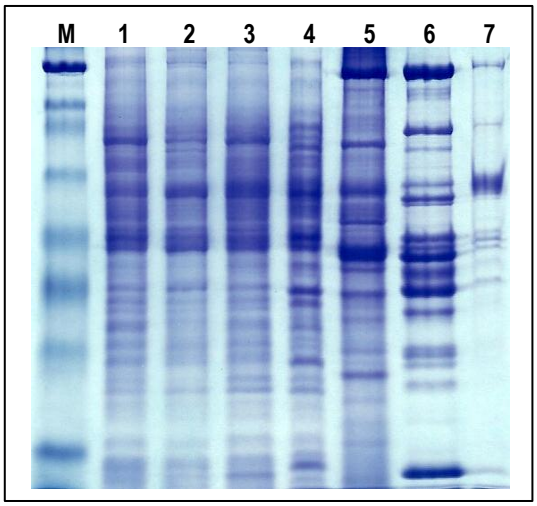

Fig. 1. SDS-PAGE gel Electrophoresis conclusion

Proteins were extracted from various cancer cell line and tissue, then analyzed on 15% SDS-PAGE.

Lane M, PRO-STAIN (I) Marker; **lane 1,** K562; **lane 2,** SNU 1; **lane 3,** SNU 5; **lane 4,** B16; **lane 5,** rat Heart; **lane 6,** cow muscle; **lane 7,** Cow subcutaneous fat

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
In case of using tissue sample		- First put tissue into a medicine pestle bowl and grind. The important thing is keep the medicine pestle bowl in refrigerator temperature.
Tiny of extraction protein. Regratable lysis.	Cell number	- Check cell vol. and a following PRO-PREP™ Solution vol.
	Lysis hour	- Check lysis method. Extend lysis time and do vortexing during extraction of protein for higher lysis efficience.
Protein degradation		- Do not leave too long in a room temperature while doing lysis with PRO-PREP™ Solution. - Check the extracted protein's keeping condition by using PRO-PREP™ Solution and this product. Keep everything in 20℃.
Too much bubble comming out during pipetting		- Do not need worry. During doing incubation or doing centrifuge for lysis it can disappear.
Freezed PRO-PREP Solution		- Check a deep-freezer temp. - If keeping temp. is lower than 20℃, it will be frozen, at this point do not have problem if you use after melt it. However if you do again and again, it might cause some problem, so keep a storing temp. as possible.
Occur clarity pellet in bellow part of PRO-PREP™ Solution	In case of too low of keeping temperature	- If we keep deep-freezer, PMSF among protease inhibitor should be clarity crystal condition. In case of this happen, after melt solution at room temperature in a minute, put ice solution for use cool temp. condition.
	Protease Inhibitor	- If we cannot keep it at -20℃, keep it separatively for need will be better for keep product's quality.
Can do IP ?		- Can do IP.

RELATED PRODUCTS

Product Name	Cat.No.
WEST-one™ Western Blot Detection System	16031 ~ 16033
WEST-ZOL™ (plus) Western Blot Detection System	16021
PRO-MEASURE™ Protein Measurement Solution	21011
SMART™ BCA Protein Assay Kit	21071
SMART™ Micro BCA Protein Assay Kit	21072
PRO-STAIN™ Prestained Protein Marker (I)	24051
SMART™ Bacterial Protein Extraction Solution	17511