

EzWay™ Phos-Pro Enrichment Kit

1. Catalog No. K07920
2. No. of Applications 10 reactions
3. Storage 1 year at room temperature

4. Contents

Component	Volume
Lysis Buffer	10ml
Dilution Buffer	30ml
Phos-Pro Solution A	3ml
Phos-Pro Solution B	5ml
Dissolving Solution	8ml
Delipidation Solution	10ml

5. Description

Protein phosphorylation is one of the most frequently occurred posttranslational modifications and plays a critical role in cellular regulatory events. Most cellular processes are in fact regulated by the reversible phosphorylation of proteins on serine, threonine and tyrosine residues. In fact, phosphorylation of proteins plays a key role in oncogenesis, cell signaling, apoptosis and immune disorders¹. Despite the importance and widespread occurrence of this modification, profiling of phosphoproteins in cells is still a challenge, due to the low copy of phosphorylated proteins in cell and the relative amount of phosphoproteins compared to unphosphorylated proteins.

Ladiolabeling by ³²P labeling is frequently used conventional method for investigation of phosphoprotein profile in conjunction with 2-DE or 1-D gel electrophoresis and autoladiogram. Alternatively, western blot analysis probed by phosphoprotein-specific antibody is also used for this purpose. Mass spectrometry has been shown to be a reliable and routine tool to identify proteins in a high throughput manner. However, the identification of phosphorylation by mass spectrometry is not a trivial matter and to this day is not routine also due to the low copy of phosphorylated proteins in cells.

EzWay™ Phos-Pro Enrichment Kit provides advanced chance in detecting protein phosphorylation in gels with non-radiolabeling method (eg. Staining with fluorescence dye) and enables quantitative comparison between cells.

EzWay™ Phos-Pro Enrichment Kit is optimized for the protein solution in denatured condition, for example, the samples prepared for 2-DE and can be adapted to native proteins. Enriched phosphorylated proteins could be detected by staining commercially available staining method using fluorescent dye² or by probing with antibodies, specific for phosphorylated proteins.

- Specificity 100%
- Selectivity >87%
- Sensitivity >93% / 50ng
- Yield >93%

For research use only; not for use as a diagnostic

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- Convenient fractionation of phosphoproteins from protein mixtures such as cell lysate or body fluids
- Isolation and concentration of low copy phosphorylated proteins in cells
- No use of IMAC column of phosphorylated antibodies
- Downstream application for 2-DE or 1-D SDS-PAGE analysis
- Enriched phosphorylated proteins detection by staining with fluorescent dye or antibody probing

6. Additional Materials Required

Methanol
Ultrapure water

7. Procedure

1. Add 300-600ul Lysis Buffer to the cells or tissue and disrupt the cells and tissue by sonication or motor driven homogenization. (Adjust the volume of Lysis Buffer in order the final concentration of extracted protein to be above 4mg/m) Vortex the cell lysate for 15min and centrifuge for 20min. at 12,000 x g and save the supernatant. Assay the protein concentration and dilute 2mg protein with Dilution Buffer to be the final volume of 3ml. (Use 5ml tube)

Alternatively, protein solution prepared for two-dimensional gel electrophoresis could be directly used for enrichment by appropriately diluted with Dilution Buffer.

2. Add 240ul of Phos-Pro Solution A and rapidly mix by vortex vigorously for a few seconds then incubate for 15min.by inverting or gentle vortexing. After subsequent adding 360ul Phos-Pro Solution B and brief mixing, incubate for 15min. by gentle vortexing then stand still for 5-10 min for the aggregated materials to be settled down. And discard about 4ml of upper clear solution.
3. Transfer the remaining aggregate suspension to 1.5ml microcentrifuge tube and centrifuge the suspension at 12,000rpm for 5min. Discard the supernatant and save the aggregate in hard pellet.
This aggregate can be stored for several days.
4. Add 0.7ml Dissolving Solution and dissolve the pellet by pipetting several times (*caution : at this time CO2 gas will be formed. Open lid and degas sufficiently before vortexing) and vortex for 5 min.
5. Add 750 ul of Delipidation solution. (methanol:chloroform=600:150) and vortex vigorously for 5 min and centrifuge at 12,000rpm for 10 min for phase separation of solution. Recover the middle phase protein disk and discard lower and upper phase solution completely. Then wash the protein disk with sufficient (~1ml) methanol for two times.
6. Dry the protein pellet in air or oven completely and dissolve the protein pellet with the solution for 2-DE electrophoresis or 1-D SDS PAGE.

8. Related Products

Cat. No.	Product	Size
K07910	EzWay™ Phos-Pep Enrichment Kit	50 reactions

