Tel: +82-54-223-2463 Fax: +82-54-223-2460
Email: alloyo02@genomine.com
http://www.genomine.com
Venture Bldg 306
Pohang Techno Park Pohang, Kyungbuk,
Korea (ROK)

INSTRUCTIONS

Phos-Pep™
Phospho-peptide enrichment kit

Product Number **P 5010**
Storage Temperature 10 to 37 °C (RT)

INTRODUCTION

Protein phosphorylation is one of the most frequently occurred posttranslational modification 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. Despite the importance and widespread occurrence of this modification, identification of protein phosphorylation site is still a challenge, due to the low copy of phosphorylated proteins in cell, even when performed on highly purified protein.

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 sites by mass spectrometry is not a trivial matter and to this day is not routine. The detection of phosphopeptides by mass spectrometry in a complex mixture, such as a tryptic mass fingerprint, is a rare occurrence. This is thought to be caused by suppression of the ionization of the mainly negatively charged phosphopeptide in the presence of a large excess of nonphosphorylated peptides[1].

This phosphopeptide enrichment kit provides highly selective enrichment of phosphopeptide from non-phosphorylated complex trytic digest of proteins and facilitates the identification of phosphopeptide by mass spectrometry.

Phosphopeptde identification relies on measuring the loss of mass. Phosphopeptides tend to lose their phosphate group as phosphoric (H_3PO_4) or phosphorous acid (HPO_3) due to metastable decay in MALDI-TOF, ESI (PSD)[2,3] ,ion trap(CID) or as phosphorous acid (HPO_3) by phosphatase[4] .

(Technical bulletin http:// www.genomine.com)

Kit contents

	50 reaction
Solution A	0.3ml
Solution B	0.4ml
Washing solution: (4×ammonium acetate buffer)	8ml
Dissolving solution: (1% phosphoric acid)	0.8ml
Phosphopeptide standard:beta casein tryptic digest 10μg	

Additional Materials Required

- C18 microtip
- Ultrapure water

Binding Capacity

In binding assays performed using this product, binding of greater than 90 pmoles of phosphopeptide per 10μl of A solution is observed in one reaction. From 1μg(45 pmole) trypsin digest of b-casein, single enrichment recover over 95% mono(2062 Da) and tetra(3124 Da) phosphopeptide.

Procedure Summary

1. Selective binding of phosphpeptide
2. Precipitation of phosphopeptide complex
3. Washing phosphopeptide aggregates
4. Dissolving of phosphopeptide aggregates

Procedure for phosphopeptide enrichment from trypsin digest

1. Prepare trypsin digest

2. Add 5μl of solution A to 10μl trypsin digest in microcentrifuge tube and vortex briefly for a few seconds then stand about 1~5min.

3. Add 5μl of solution B to the previous mixture and vortex well to disperse the aggregate to homogeneous cloudy suspension. Stand at least 30min until the cloudy aggregate settle down and disappear.(When cloudy aggregate was not disappeared within 30 min., stand prolonged time till the aggregate disappear completely.)

4. Discard solution to the last drop with pipet tip. (Phosphopeptide aggregates was stuck to the wall and remained coated in microcentrifuge tube)

This aggregate can be stored for several days.

5. Add 50μl of 1X washing solution(dilute stock solution four fold) and vortex briefly for a few seconds then discard washing solution completely.

6. Add 10μl dissolving solution and stand at least 5min. (You can see sometimes some bubble gas formed along with the surface of microcentrifuge tube)

7. Vortex briefly for a few seconds and,if necessary, dissolve the remaining crystals with pipetting.

8. Desalt or concentrate the solution with C18 microtip column for mass spectrum analysis.

Optimization of Results

When peptide solution contains high salt, dilute the solution below 100mM of salt prior to enrichment to obtain better result.

References

1. Joerg R., et al., Proteomics, 4, 3686–3703 (2004)

2. Metzger, S. and Hoffmann, R. J Mass Spectrom 35, 1165–1177 (2000).

3. Hoffmann, R. et al. J Mass Spectrom 34, 1195–1204 (1999).

4. Akira Yamagata, et al., Proteomics, 2, 1267–1276 (2002)

Related Products Product Code
Phos-pro Phosphoprotein enrichment kit P5012