2010 Products Catalog of Genomine,Inc.

Thy best research partner in proteomics

From sample preparation to data mining.....

Pursuiting excellence and superior service,
we are dedicated to providing you with uppermost results.
Building Values through Genomine Proteomics Services
for research collaboration and proteome analysis service.

GENOMINE
For better life through Genomics

Venture Bldg 307 Pohang TechnoPark, 601, Jigok, Pohang, Gyeongbuk, 790-834 KOREA.
TEL : +82-54-223-2464 FAX : +82-54-223-2460 E-mail : gmkim@genomine.com

http://www.genoproteom.com

INDEX

Overview, User manual, and Technical bulletin

1. Overview

2. User manual & Technical bulletin

GENOMINE

Venture Bldg 307 Pohang TechnoPark, 601, Jigok, Pohang, Gyeongbuk, 790-834 KOREA.
TEL : +82-54-223-2464 FAX : +82-54-223-2460 E-mail : gmkim@genomine.com

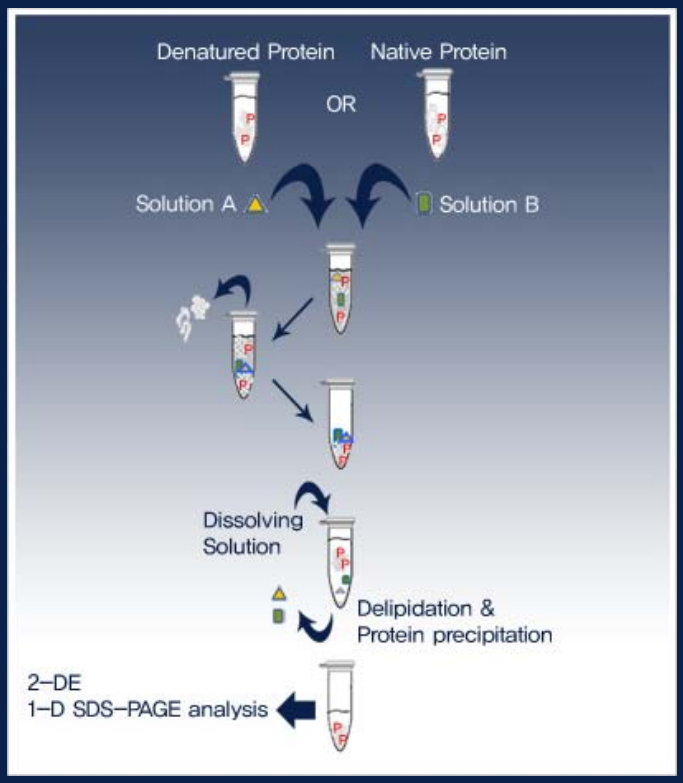
http://www.genoproteom.com

Phosphoprotein enrichment kit – PhosPro™

Highly selective and enrichment of phosphoprotein from complex mixtures of cell lysate

Highlights of PhosPro™ kit

- Phosphoprotein specific
- Sensitive
- Convinient
- Reproducible
- Enrichment from native or denature protein solution



Evaluation category	Specification
Specificity	100%
Selectivity	>87%
Sensitivity	>93% / 50ng
Yield	>93%

Ordering information

Product	Description	Cat. No.
PhosPro™ kit	1 box (5 Reaction)	P5012-5
PhosPro™ kit	1 box (10 Reaction)	P5010-10

Kit contents 10 reactions

- Lysis buffer 10ml x 1
- Dilution buffer 20ml x 1
- Solution A 3ml x 1
- Solution B 5ml x 1
- Dissolving Solution 8ml x 1
- Delipidation Solution 10ml x 1

Procedure Summary

1. Native & denatured Protein extraction
2. Phosphoprotein specific complex forming
3. Precipitation of phosphoprotein complex
4. Dedelipidation and recovering of phosphoprotein

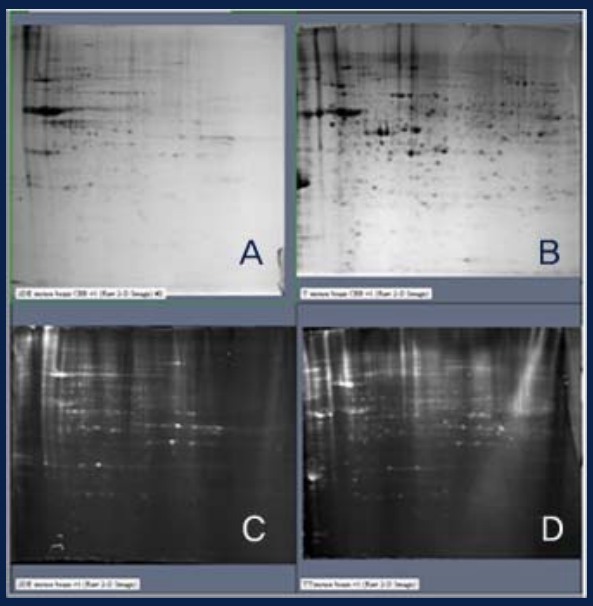


Fig. 2-DE analysis of enriched phosphoprotein fraction from total denatured(A,C) & native protein(B,D) stained with CBB(A,B), and stained with ProQ Diamond staining(C,D)

GENOMINE
For better life through Genomics
Venture Bldg 307 Pohang TechnoPark, 601, Jigok, Pohang, Gyeongbuk, 790-834 KOREA.
TEL : +82-54-223-2464 FAX : +82-54-223-2460 E-mail : gmkim@genomine.com

PhosPep™ – Phosphopeptide enrichment kit

Isolation and enrichment of phosphopeptide from complex mixtures of enzyme digest of phosphorylated proteins

✔Highlights of PhosPep™ kit

- Specific
- Sensitive
- Fast
- Non-IMAC

Kit contents 50 reactions

- Solution A
- Solution B
- Washing solution:
  4×ammonium acetate buffer
- Dissolving solution:
  1% phosphoric acid
- Phosphopeptide standard:
  beta casein tryptic digest

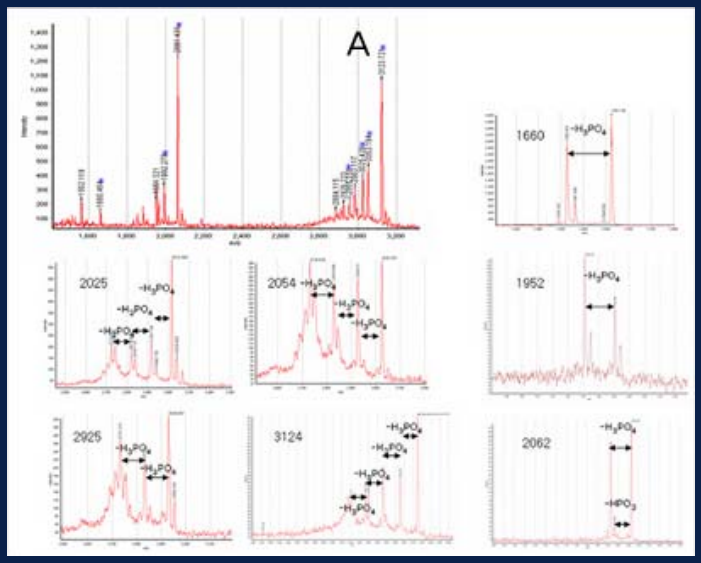
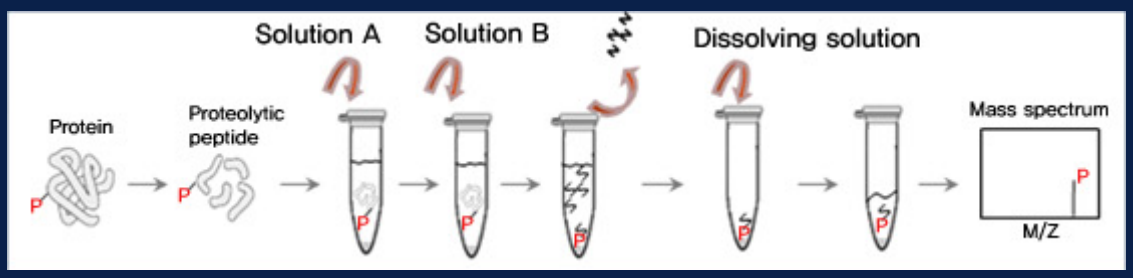


Fig. Identification of phosphopeptide enriched from beta casein trypsin digest by PSD(post source decay) using MALDI-TOF. Panel A represents the MALDI-TOF spectrum of enriched phosphopeptide from beta casein trypsin digest. Blue asterisk represent the enriched phosphopeptides. Rest of the seven spectrums represent the PSD spectrum of enriched phosphopeptide.

Procedure Summary

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



Ordering information

Product	Description	Cat. No.
PhosPep™ kit	1 box (50 Reaction)	P5010

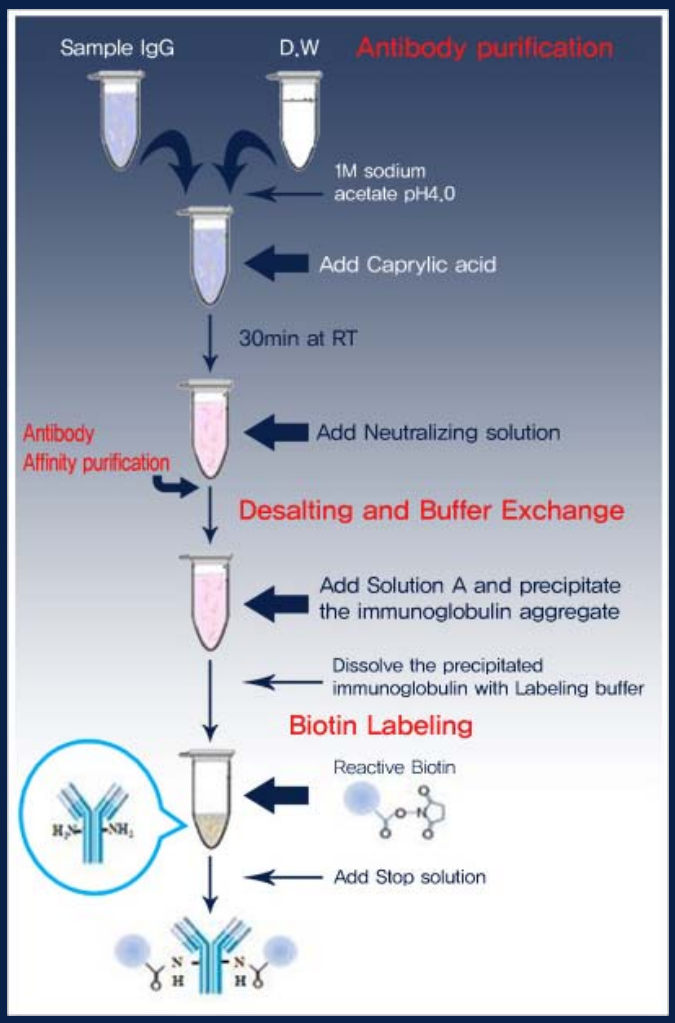
GENOMINE
For better life through Genomics
Venture Bldg 307 Pohang TechnoPark, 601, Jigok, Pohang, Gyeongbuk, 790-834 KOREA.
TEL : +82-54-223-2464 FAX : +82-54-223-2460 E-mail : gmkim@genomine.com

Antibody biotin conjugation kit

One stop labeling of Antibody Purification to Biotinylation

✔Highlights of Biotinylation kit

- **Quick**
  Only 1 hour to get conjugates
- **Easy**
  No filtration tube required
- **Reliable**
  High recovery & consistent
- **Efficient**
  Applicable for 20ug to 10mg IgG



Kit contents 10 reactions

- **Antibody purification**
  - 1M Sodium Acetate pH4.0 1ml x 1
  - Carprylic Acid 0.5ml x 1
  - Neutralizing Buffer 1ml x 1
- **Desalting & buffer exchange**
  - Solution A 8ml x 1
  - Labeling Buffer 6ml x 1
- **Biotin labeling**
  - Reactive Biotin x 1
  - Stop Solution 0.3ml x 1

Procedure Summary

1. Antibody Purification
2. Desalting and Buffer Exchange
3. Biotin labeling
4. Stopping and Storage

Ordering information

Product	Description	Cat. No.
Antibody biotin conjugation kit	1 box (10 Reaction)	P5014

GENOMINE
For better life through Genomics
Venture Bldg 307 Pohang TechnoPark, 601, Jigok, Pohang, Gyeongbuk, 790-834 KOREA.
TEL : +82-54-223-2464 FAX : +82-54-223-2460 E-mail : gmkim@genomine.com

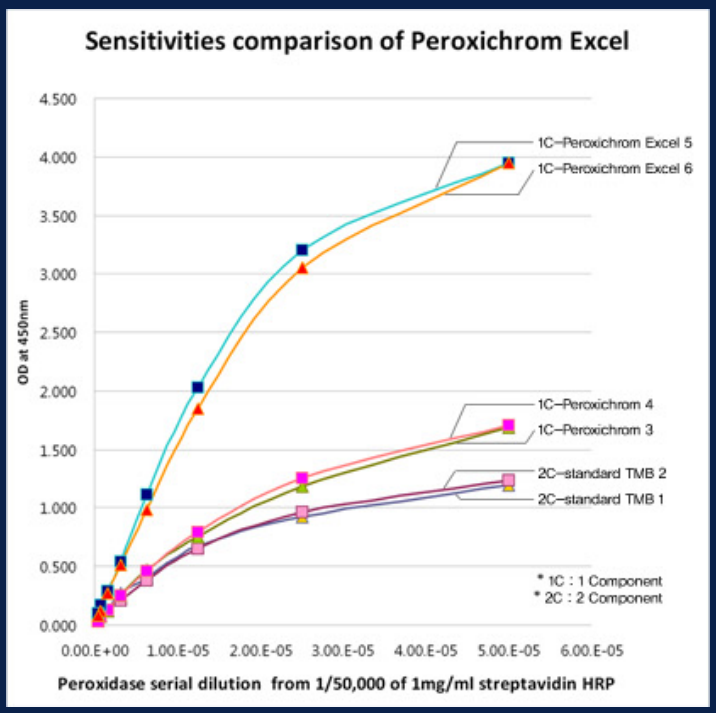
Peroxichrome™, Peroxichrome Excel™

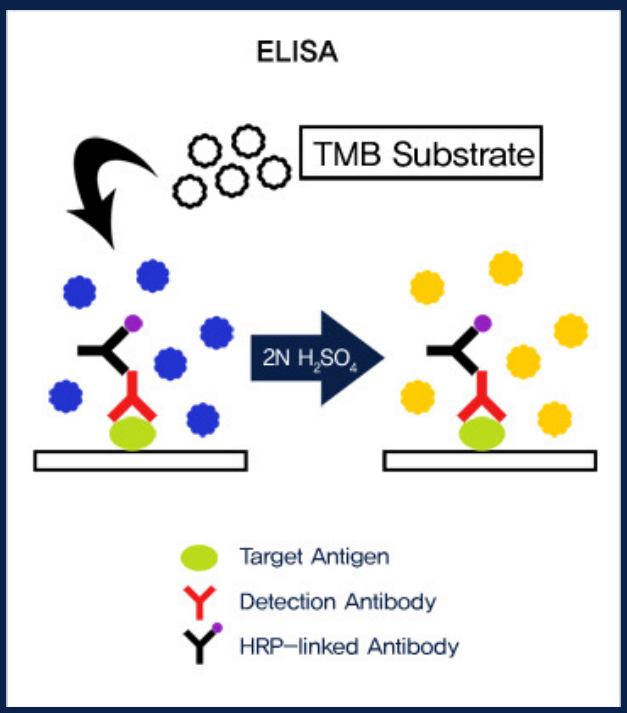
TMB Peroxidase substrate solution (1-Component) for ELISA

Highlights of TMB substrate kit

- Ready to use single component
- Highest sensitivity
- Sufficient dynamic range
- Easy to use
- Noncarcinogenic
- No hydrogen peroxide required
- No DMF or DMSO present in the reagent
- Stable at RT
- Ease of shipping
- Pricing

TMB substrate(3,3′,5,5′-tetramethylbenzidine) is a chromogen that yields a deep blue color when oxidized with hydrogen peroxide (catalyzed by HRP). The color then changes to yellow with the addition of 2N H2SO4 with maximum absorbance at 450 nm.Our TMB Substrates(Peroxichrom™, peroxichrom Excel™) are one-component substrates that require no pre-paration before using, stable and sensitivity. Also (Peroxichrom™, peroxichrom Excel™) contain no solvents or organics such as DMF, DMSO, methanol so there is no issue of safety with user.





Ordering information

Product	Description	Cat. No.
Peroxichrom™	1 box (100ml X 4)	D5015-400
Peroxichrom™	100ml	D5015-100
Peroxichrom Excel™	1 box (100ml X 4)	D5016-400
Peroxichrom Excel™	100ml	D5016-100

GENOMINE
Venture Bldg 307 Pohang TechnoPark, 601, Jigok, Pohang, Gyeongbuk, 790-834 KOREA.
TEL : +82-54-223-2464 FAX : +82-54-223-2460 E-mail : gmkim@genomine.com

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

INSTRUCTIONS

PhosPro™
Phosphoprotein enrichment kit

Product Number **P 5012**
Store at RT

This phosphoprotein enrichment and exclusion of unphosphorylated proteins provides advanced chance in detecting protein phosphorylation in gels with non-radiolabeling method(eg. Staining with fluorescence dye) and enables quantitative comparison between cells.

INTRODUCTION

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 disorders[1]. 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.

Radiolabeling by ^{32}P labeling is frequently used conventional method for investigation of phosphoprotein profile in conjunction with 2-DE or 1-D gel electrophoresis and autoradiogram. 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.

Kit contents

	10 reactions
LYSIS BUFFER	10ml x 1
DILUTION BUFFER	30ml x 1
Native Homogenation Buffer	30ml x 1
SOLUTION A	3ml x 1
SOLUTION B	5ml x 1
DISSOLVING SOLUTION	8ml x 1
DELIPIDATION SOLUTION	10ml x 1

Additional Materials Required

- Methanol
- Ultrapure water

Detecting Phosphorylated Proteins

This phosphoprotein enrichment kit was optimized for the protein solution in denatured condition, for example, the samples prepared for 2-DE and can be applied to native proteins. Enriched phosphorylated proteins could be detected by staining commercially available staining method using fluorescent dye[2] or by probing with antibodies, specific for phosphorylated proteins.

Procedure Summary

1. Protein extraction
2. Phosphoprotein specific complex forming
3. Precipitation of phosphoprotein complex
4. Dedelipidation and recovering of phosphoprotein

Procedure for phosphoprotein enrichment from cell lysate
(Denatured protein condition)

1. Add 300~600μl LYSIS SOLUTION to the cells or tissue and disrupt the cells and tissue by sonication or motor driven homogenation. (Adjust the volume of LYSIS SOLUTION 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 SOLUTION 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 SOLUTION.

2. Add 240μl of SOLUTION A and rapidly mix by vortex vigorously for a few seconds then incubate for 15min.by inverting or gentle vortexing. After subsequent adding 360μl 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 CO_2 gas will be formed. Open lid and degas sufficiently before vortexing) and vortex for 5 min.

5. Add 750 μl of delipidation soln. (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.

Alternative procedure for phosphoprotein enrichment from cell lysate
(Native protein condition)

If you want to isolate the phosphorylated protein from cell lysate in native conformation of proteins, omit step 1 using LYSIS SOLUTION. Instead, prepare the cell lysate with Native Homogenation Buffer or appropriate buffer solution except solution including phosphate.

1. Add 1~3ml Native Homogenation Buffer to the cells or tissue and disrupt the cells and tissue by sonication or motor driven homogenation in order the final concentration of extracted protein to be 2~30mg/ml. Centrifuge for 20min. at 12,000 x g and save the supernatant. (Use1 or 5ml tube)

2. Add 80μl of SOLUTION A per 1ml protein solution and mix gently for 15min by inverting or gentle vortexing. After subsequent adding 120μl SOLUTION B per 1ml original solution, incubate for 15min by gentle inverting then centrifuge briefly for 5~10 min for the aggregated materials to be settled down. And discard upper clear solution.

3. Add one fourth volume of Native Homogenation Buffer to the pelletted phosphoprotein complex and wash the residual non-phosphorylated protein solution by resuspension the pellet and recover the pellet

by brief centrifugation. Repeat this washing one time and save the aggregate in hard pellet.

4. Add 0.7ml DISSOLVING SOLUTION and dissolve the pellet by pipetting several times(*caution : at this time CO_2 gas will be formed. Open lid and degas sufficiently) and stand for 10 min. the solution to be clear.

This solution contains enriched phosphoprotein in 250mM salt solution containing EDTA. Dialysis the enriched phosphoprotein solution with appropriate buffer solution.

Or skip to the next step for preparation of 1D or 2D electrophoresis samples.

5. Add 750 μl of delipidation soln. (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.

Optimization of Results

When you start with cell lysate at higher concentration and smaller volume of proteins, use SOLUTION A and SOLUTION B, 80μl and 120μl per 1ml protein solution respectively.

References

1. Philip Cohen, *Eur. J. Biochem*., 568*,* 2001-2010 (2001)

2. Alein, L. *et al*. Proteomics, 6, 2157–2173 (2006).

Related Products Product Code

Phospep Phosphopeptide enrichment kit

Technical bulletin
Tel: +82-54-223-2463 Fax : +82-54-223-2460
http://www.genomine.com
venture Bldg 306 Pohang techno park Pohang, kyungbuk, Korea(ROK)

Enrichment and identification of phosphopeptide using PhosPro™

Introduction

PhosPro™, phosphoprotein enrichment kit, was developed to fractionate phosphorylated proteins from protein mixtures, such as cell lysate or body fluids and it is efficient to isolate and concentrate low copy phosphorylated proteins in cells. This kit utilized proprietary phosphoprotein precipitation method instead using column or bead such as IMAC column or immobilized anti-phophoprotein antibody beads. Then in addition to its specificity for isolation of phosphoproteins, it provides simple and convenient method for phosphoprotein fractionation and all processes are to be done with multi parallel samples in each one tube. This kit was designed to use denaturant and detergent solution as the starting material of protein extraction in order phosphoprotein isolation not to be prevented by possible steric hindrance of phosphorylated moiety of proteins and not to be omitted by difficulty in solubilization of phosphoproteins embedded in membrane fraction or cell debris.

Strategy

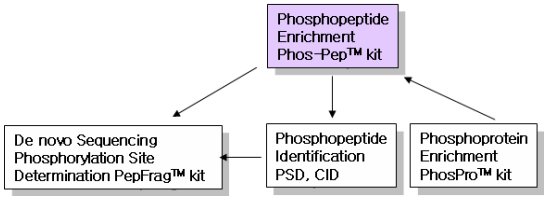


Fig.1. Phosphoprotein enrichment using PhosPro™. Protein phosphorylation could be identified by phosphoprotein specific enrichment in conjunction with phosphoprotein specific staining or MS-based phosphopeptide identification and phosphorylation site determination.

Materials & Methods

Materials

PhosPro™ kit contents

LYSIS BUFFER

DILUTION BUFFER

NATIVE HOMOGENATION BUFFER

SOLUTION A

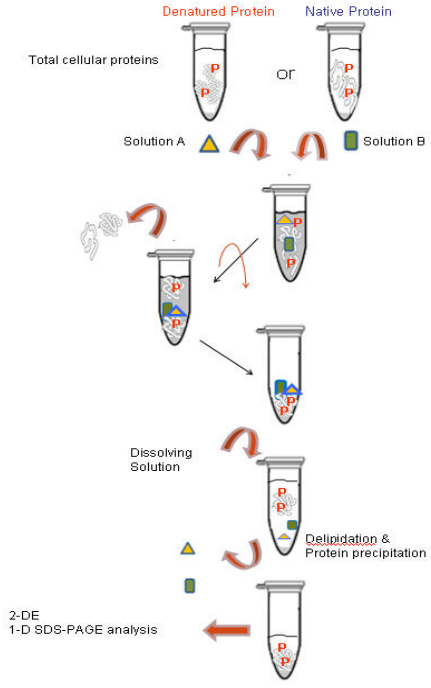
SOLUTION B

DISSOLVING SOLUTION

DELIPIDATION SOLUTION

Procedure Summary

1. Native & Denatured Protein extraction
2. Phosphoprotein specific complex forming
3. Precipitation of phosphoprotein complex
4. Delipidation and recovering of phosphoprotein



Additional Materials Required

. Methanol
. Ultrapure water

Detection of Phosphorylated Proteins

This phosphoprotein enrichment kit was 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 dye[1] or by probing with antibodies specific for phosphorylated proteins.

Results and Discussion

Phosphoprotein enrichment from Saccharomyces cerevisiae protein extract

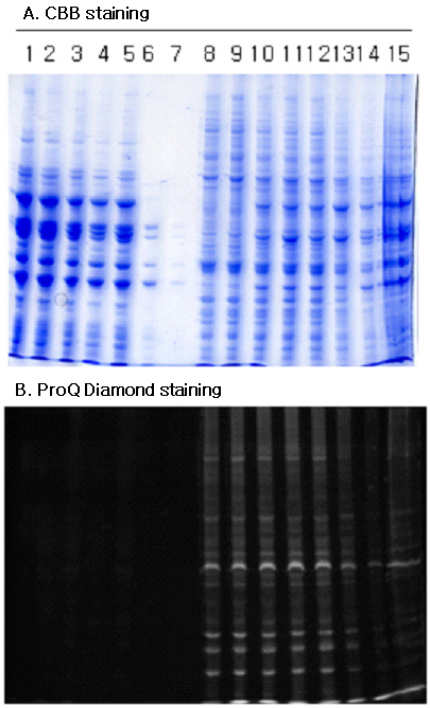


Fig.2. SDS-PAGE analysis of enriched phosphorylated proteins by PhosPro™. A: Coomassie Brillant Blue(SIGMA) staing, B:ProQ Diamond(Invitrogen) staining. Lane 1~7 ; The supernatant containing unphosphorylated proteins discarded in step3(See Procedure Summary above), Lane 8~15 ; enriched fraction containing phosphorylated proteins. Independent seven trials for phosphoprotein enrichment were performed and analysed by SDS-PAGE.

In order to evaluate the performance of PhosPro™, yeast protein extract was used. The resulting enriched phosphorylated protein was analysed with 1-D(Fig.2) or two-dimensional gel electrophoresis(2-DE, Fig.3)

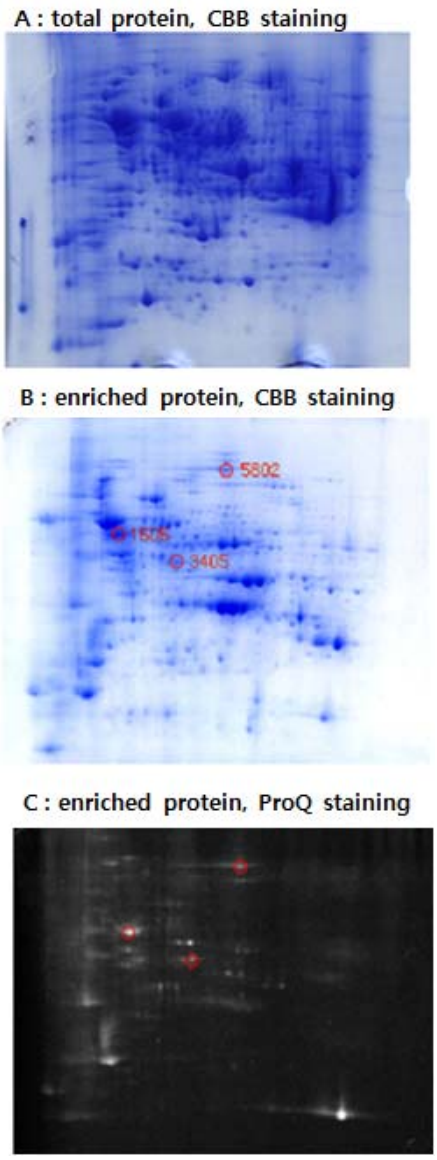


Fig.3. 2-DE analysis of total protein(A), enriched phosphoprotein fraction stained with CBB(B), and stained with ProQ Diamond staining(C)

As shown in Fig.2. and Fig.3. most of the proteins stained with ProQ Diamond was detected in enriched phosphoprotein fraction. This result represent that the PhosPro™ is highly specific for phosphoprotein fractionation.

Phosphoprotein enrichment from mouse brain native protein extract

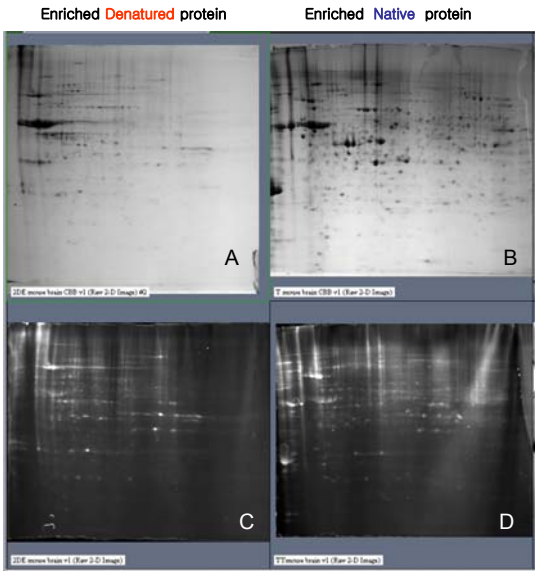


Fig.4. 2-DE analysis of enriched mouse brain phosphoprotein fraction from total denatured(A,C) & native protein(B,D) stained with CBB(A,B), and stained with ProQ Diamond staining(C,D)

In order to evaluate the performance of PhosPro™, mouse brain denatured(A,C) and native protein(B,D) extract was used as a starting material. The resulting enriched native phosphorylated protein was analysed using 2-DE (Fig.4)
As shown in Fig.4.D most of the proteins stained with ProQ Diamond in denatured protein fraction was detected in enriched phosphoprotein fraction from total native protein. But native phosphoprotein fraction contains more non-phosphorylated proteins(not stained with ProQ Diamond), which is assumed as subunit or proteins interacting with phosphorylated proteins than denatured phosphoprotein fraction.
As a result, PhosPro™ is applicable to both denatured rather pure phosphoprotein fraction and active phosphylated protein fractionation.

Phosphoprotein identification by mass spectrometry

The protein spot enriched and stained with ProQ Diamond and identified as a phosphoprotein(spot N. 3405 in Fig.3. B and C) was further confirmed by mass spectrometry. Protein was identified by MALDI-TOF-based peptide mass fingerprinting. Its phosphopeptide was enriched by PhosPep™ and identified with MALDI-PSD by detecting the loss of phosphoprous group(98Da) from mother phosphopeptide(m/z 2900.392).

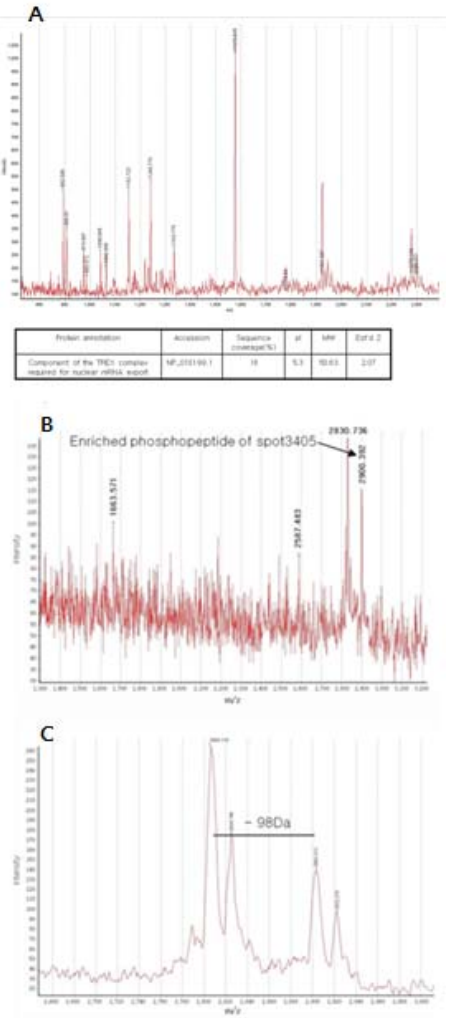


Fig.5. Identification of enriched phosphoprotein by mass spectrometry.

Phosphoprotein identification by dephosphorylation using λPPase

The phosphoproteins were enriched by PhosPro™ from cell lysate of h460 lung cancer cell line. The enriched protein fraction which was stained with phosphoprotein staining and presumed to be the phosphoproteins, was confirmed whether the staining was derived from the phosphate moiety on the proteins by examining the

changes after treatment of phosphatase. The λPPase was used as a phosphatase as previously described[2].

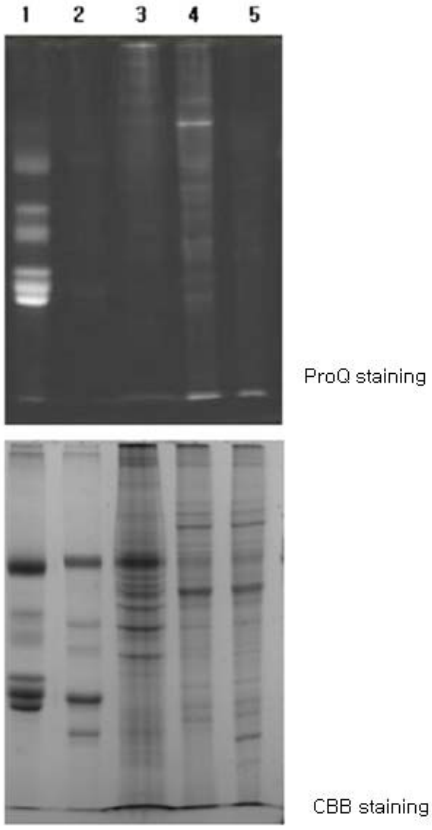


Fig.6. Phosphoprotein staining of enriched phosphoproteins and dephosphorylated proteins. Lane1: standard phosphoproteins, lane2, standard phosphoprotein treated with λPPase, lane3: supernatant fraction remained from phosphoprotein enrichment, lane4: phosphoprotein fraction enriched by PhosPro™, lane5:phosphoprotein fraction treated with λPPase

As shown in Fig.5 the proteins in enriched phosphoprotein fraction was stained with phosphoprotein staining whereas the same proteins treated with phosphatase, λPPase, was not stained with phosphoprotein staining but only with CBB staining. This result represent that the proteins stained with phosphoprotein staining was phosphate group specific and the PhosPro™ isolate the phosphoproteins from cell extract in a specific manner.

Enrichment of low abundant phosphoproteins from lung cancer cell lines and lung cancer tissues

In 2-DE gel analysed using 300ug proteins of total cell lysate of lung cancer cell lines(h460), about 35 protein spots was stained with phosphoprotein staining. When the phosphoproteins(300ug) were enriched from total protein extract of lung cancer cell line, 198 protein spots were detected as a phosphoproteins.(Fig.6.B).

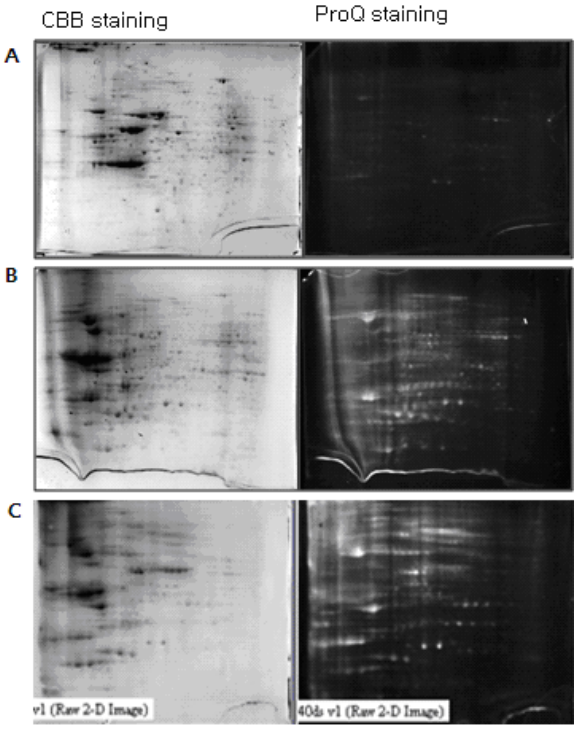


Fig.7. 2-DE gel analysis of total extract of lung cancer cell line(A), enriched phosphoprotein pool from the extract of human lung cancer cell line(B) and enriched phosphoprotein pool from the extract of human lung cancer tissues(c)

This result was represented in human lung cancer tissues which is composed more complex composition of cell types and body fluids.

As a result from the evaluation with phosphoprotein standards, α-casein, β-casein, pepsin, ovalbumin and phosvitin, PhosPro™ technology showed phosphoprotein specific, sensitive and high yield effective fractionation capabilities.(Table.1)

Table.1 Phosphoprotein fractionation capability of PhosPro™

Evaluation category	Specification
Specificity	100%
Selectivity	>87%
Sensitivity	>93% / 50ng
Yield	>93%

References

1. Alein, L. *et al*. Proteomics, 6, 2157–2173 (2006).
2. Akira Yamagata, et al., Proteomics, 2, 1267–1276 (2002)

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
Solution B
Washing solution:4×ammonium acetate buffer
Dissolving solution:1% phosphoric acid
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

Technical bulletin
Tel: +82-54-223-2463 Fax : +82-54-223-2460
http://www.genomine.com
venture Bldg 306 Pohang techno park Pohang, kyungbuk, Korea(ROK)

Enrichment and identification of phosphopeptide using Phos-Pep™

Introduction

Phos-pep™ was designed to ensure the charactrization of phosphopeptides. Investigators to characterize the phosphoproteins by mass spectrometry hampered by the low abundance of phosphoprotein and the suppression of ionization of phosphopeptide in mass spectrometry resulting in failure to obtain sufficient signals. Phos-Pep™ facilitates the isolation and enrichment of phosphopeptide from complex mixtures of trypsin digest of phosphorylated proteins.

Strategy

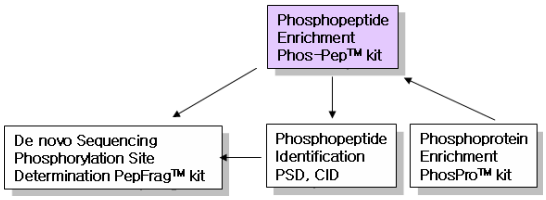


Fig.1. Phosphopeptide enrichment using Phos-Pep™ in conjunction with MALDI-TOF-based CAF sequencing or MS/MS for identification of phosphopeptide and phosphorylation site determination.

Materials & Methods

Materials

Phos-pep™kit contents

Solution A
Solution B
Washing solution : ammonium acetate stock solution
Dissolving solution : 1% phosphoric acid
Phosphopeptide standard : beta casein tryptic digest 10μg

Procedure Summary

1. Selective binding of phosphpeptide
2. Precipitation of phosphopeptide complex
3. Washing phosphopeptide aggregates
4. Dissolving of phosphopeptide aggregates
5. Desalting and concentration
6. Characterization of phosphopeptide

Results and Discussion

Phosphopeptide enrichment from beta casein

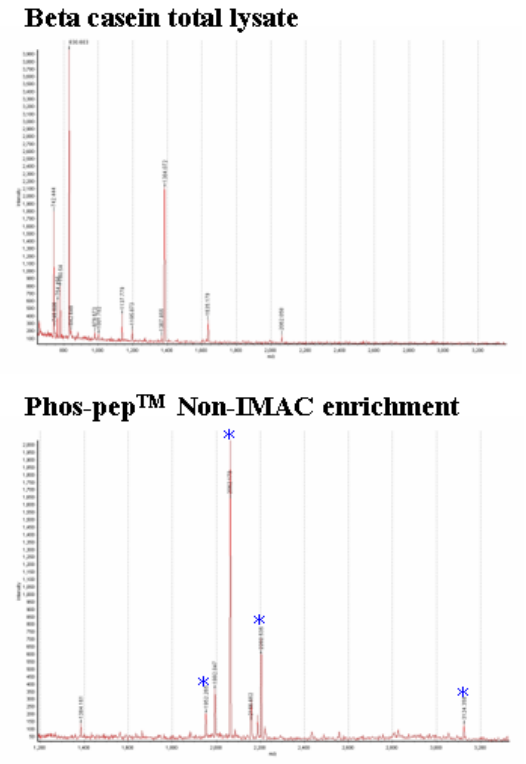


Fig.2. MALDI-TOF mass spectrum of beta casein trypsin digest(upper) and enriched phosphopeptide from beta casein trypsin digest using Phos-Pep™ (lower). Blue asterisk represent the enriched phosphopeptides.

Phosphopeptide identification

Beta casein was used to examine the capacity of Phos-Pep^{TM}, non-IMAC phosphopeptide enrichment kit. Beta casein (purchased from Sigma, Cat.No.C6905) was digested with trypsin and phosphopeptide was enriched using Phos-Pep^{TM} and peptide map was measured. Phosphopeptide which was not shown in total digest of beta casein(Fig.2.) was detected in enriched fraction(Fig.2. lower panel, Fig.3. A). Among these phosphopeptide, mono phosphopeptide(2062) and tetra-phosphopeptide(3124) was originated from beta casein. Some phosphopeptide, 1660, and 1952, seems to be originated from contaminated alpha casein, because this phosphopeptide is a major component of commercially available alpha casein phosphopeptide(see Fig.4.). Some peptides, ranging 2884 to 3054, seems to be the derivatives of tetra-phophopeptide of 3124, which have differential mass according to the status of the number of phosphorylation site. Some of phosphopeptide was identified by PDS of MALDI-TOF by detecting the mass loss of phosphoric or phosphorous acid (Fig.3.).

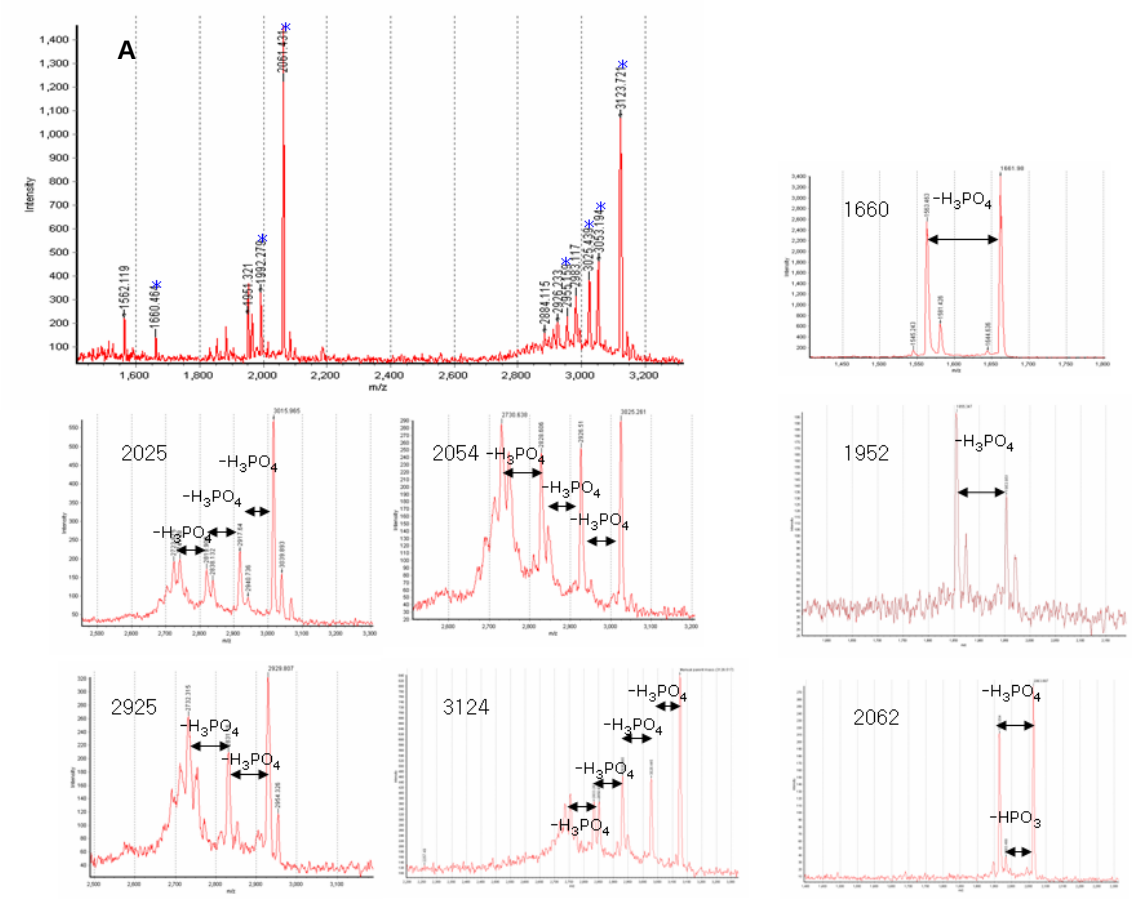


Fig.3. Identification of phosphopeptide enriched from beta casein trypsin digest by PSD(post source decay) using MALDI-TOF. Panel A represents the MALDI-TOF spectrum of enriched phosphopeptide from beta casein trypsin digest. Blue asterisk represent the enriched phosphopeptides. Rest of the seven spectrums represent the PSD spectrum of enriched phosphopeptide.

Specificity and sensitivity of phosphopeptide enrichment

Heterogeniety of phosphorylation site in peptide sequence could affect the efficiency of phosphopeptide enrichment. We examined the specificity of Phos-Pep™ kit for phosphopeptide from known phosphoprotein as a model peptide, such as alpha casein, ovalbumin, pepsin and phosvitin. As shown in Fig.4., Phos-Pep™ isolated the phosphopeptide from most of the tryptic digest of model protein used and characterized as a phgosphopeptide by PSD using MALDI-TOF. The sensitivity of capability of Phos-Pep™ was also tested. From one microgram trypsin digest of beta casein, which is equivalent to 45 pmole of each digested peptide, to 175 fmole of 256-fold diluent, the capacity of enrichment was tested(Fig.5.)

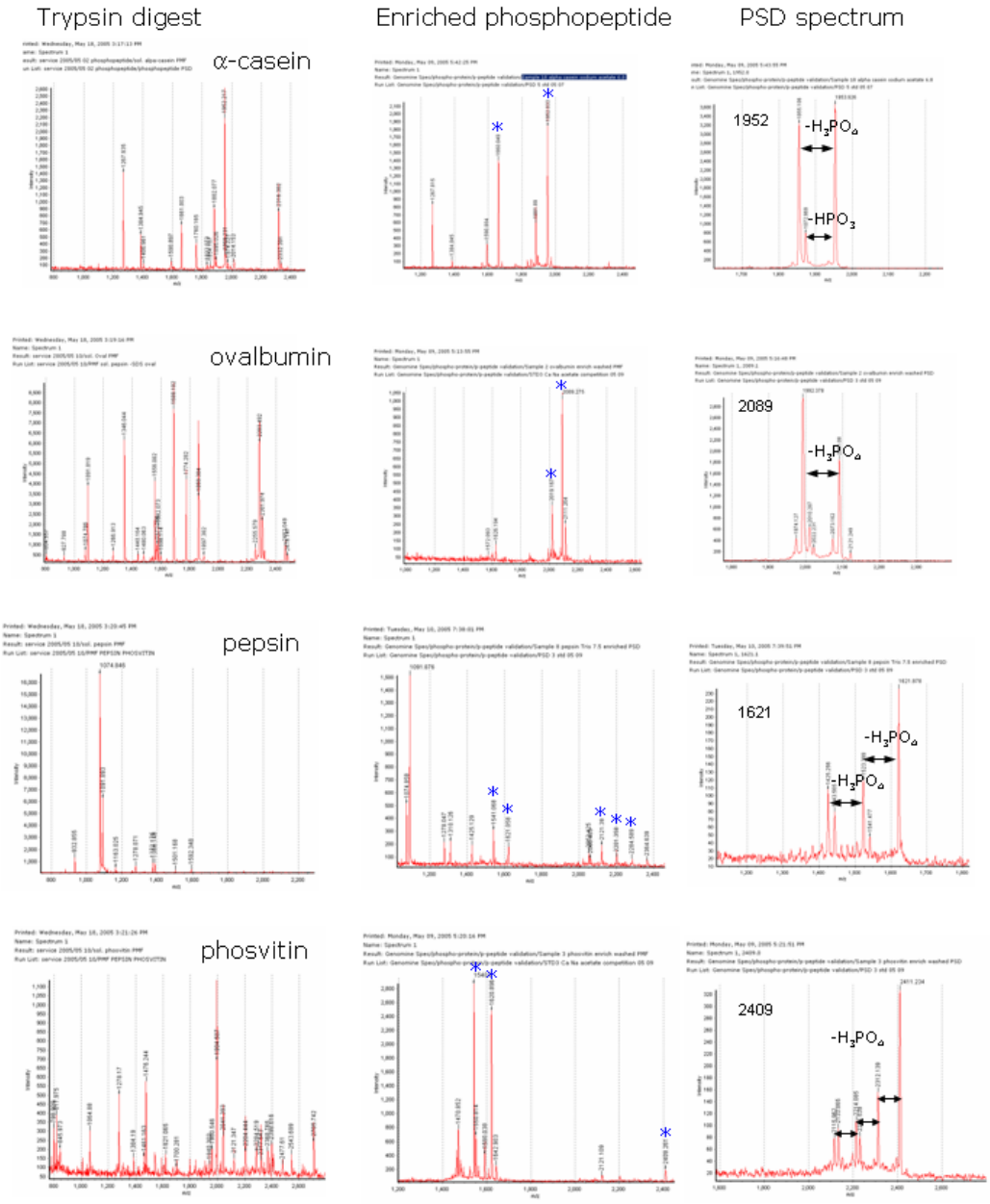


Fig.4. Phosphopeptide enrichment from trypsin digest of phosphoprotein

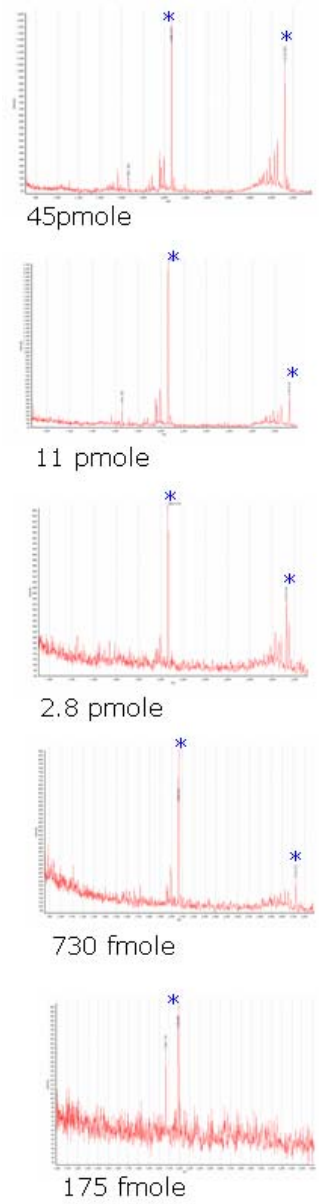


Fig.5. Sensitivity of enrichment of phosphopeptides.

Phosphorylation site determination from enriched phosphopeptide

Beta casein 2062 phosphopeptide CAF sequencing

FQpSEEQQQTE

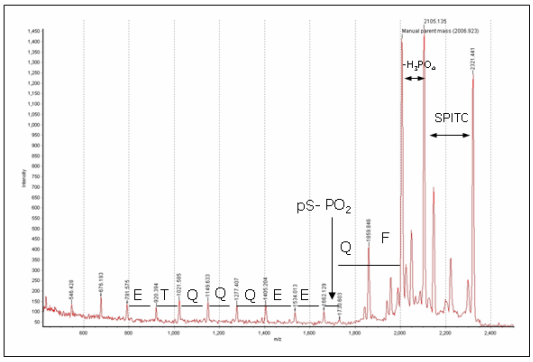
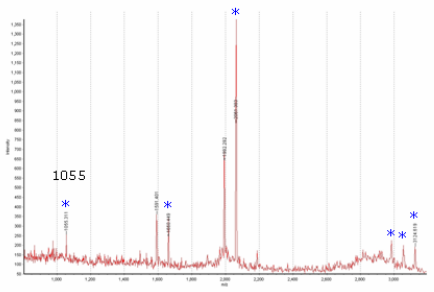


Fig.6. Determination of phosphorylation site of beta casein phosphopeptide enriched using Phos-Pep™ followed by chemical assisted fragmentation (CAF).

Phosphopeptide enrichment from beta-casein trypsin digest mixed with pTyr synthetic phosphopeptide



PSD spectrum of 1055(m/z)
(**pTyr-Asp-Leu-Leu-Leu-Glu**)

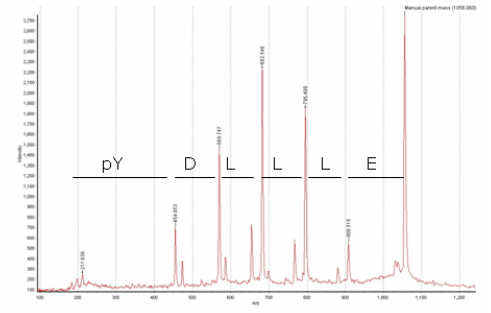


Fig.7. Enrichment and phosphorylation site determination of phosphopeptide containing phosphotyrosin amino acid residue.

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

Antibody Biotinylation Kit

Product Number **P 5013**
Store at RT

INTRODUCTION

Biotin is used in two-step detection systems in concert with conjugated avidin. Biotin is typically conjugated to proteins via primary amines (i.e., lysines). Usually, between 3 and 6 biotin molecules are conjugated to each antibody.

The entire conjugation can be performed in about an hour. In general, you will need to have a solution of your antibody at a concentration (optimally) of at least 2 mg/ml. The extent of biotin conjugation to the antibody may depend on the concentration of antibody in solution; for consistent conjugations, use a consistent concentration. This product provides convenient and efficient method for removing salt and amine compound interfering biotin coupling reaction from antibody solution.

Kit contents

	10 reactions
1M Sodium Acetate pH4.0	1ml x 1
Carprylic Acid	0.5ml x 1
Neutralizing Buffer	1ml x 1
Solution A	8ml x 1
Labeling Buffer	8ml x 1
DMSO	0.1ml x 1
Reactive Biotin	1
Stop Solution	0.3ml x 1
Solution B	8ml x 1

Additional Materials Required

- Ultrapure water
- 80% Glycerol (Optional)

Alternatives For Reactive Biotin

Reactive Biotin, NHS-Biotin, could be replaced by commercially available activated biotin,

Procedure Summary

1. Antibody Purification
2. Desalting and Buffer Exchange
3. Biotin labeling
4. Stopping and Storage

Procedure

Antibody Purification by Carprylic Acid precipitation

1. Add 500μl of DW to 500μl serum and acidify by adding 60μl of 1M sodium acetate pH 4.0

2. Slowly (drop-wise) add 37μl (20ul for rat or mouse serum) of caprylic acid and continue stirring for 20 min at room temperature.

3. Centrifuge at 12,000xg for 10min. and carefully remove and save the supernatant.

4. Adjust the pH 7.5~8.5 of supernatant antibody solution by adding 50μl of neutralizing solution and if necessary, centrifuge the solution at 8,000xg for 5min. and discard precipitate.

Alternatively start here if you have your own affinity purified IgG.

Desalting and Buffer Exchange

5. Add half volume of Solution A (0.5ml) and mix thoroughly by gentle inverting then stand for 10 min

6. Precipitate the immunoglobulin aggregate by centrifugation at 12,000xg for 5min and remove completely the turbid supernatant.

7. Dissolve the precipitated immunoglobulin with 300μl Labeling Buffer (to be approximate 2mg/ml IgG).

8. If there is any insoluble material discard it by high speed centrifugation for 10 min.

Biotin Labeling

9. Dissolve the Reactive Biotin with 60 μl DMSO(**10mM in DMSO**). Aliquote and store at -70oC. Add 2μl Reactive Biotin solution per 100μl IgG solution and incubate 30min at room temperature.

10. At the end of the incubation, add 2μl Stop Solution and subsequently add half volume of Solution B to aggregate the biotin labeled IgG and remove the residual reactive biotin. Stand this suspension at 4°C for 20min.

11. Centrifugation the suspension at 12,000xg for 10min and dissolve the aggregated IgG with equal volume of Labeling Buffer(phosphate buffer). Add 80% glycerol and store at -20℃.

Tel : +82-54-223-2464
E-mail : gmkim@genomine.com
Venture Bldg 307 Pohang Techno Park 601
Pohang, Gyeongbuk, Korea

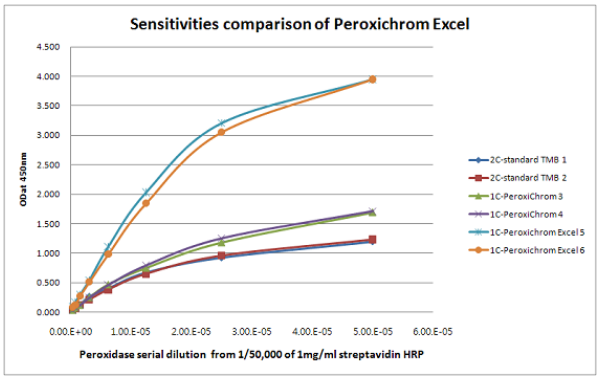
Peroxichrom™,Peroxichrom Excel™

TMB peroxidase substrate solution (1-component) for ELISA

Cat. # : D5015-100(Peroxichrom™ : 100ml)
D5015-400 (Peroxichrom™ : 400ml)
D5016-100 (peroxichrom Excel™ : 100ml)
D5016-400 (peroxichrom Excel™ : 400m)

Highlights of TMB substrate

- Ready to use single component
- Highest sensitivity
- Sufficient dynamic range
- Easy to use
- Noncarcinogenic
- No DMF or DMSO present in the reagent
- Stable at RT
- Easy to transport



Description :

TMB substrate(3,3'.5,5'-tetramethylbenzidine) is a chromogen that yields a deep blue color (maximum absorbance at 605nm or 650nm) when oxidized with hydrogen peroxide(catalyzed by HRP). The color then changes to yellow with the addition of 2N H_2SO_4 with maximum absorbance at 450 nm. Our TMB Substrates(Peroxichrom™, Peroxichrom Excel™) are one-component substrates that require no preparation before using, stable and sensitivity. Also Peroxichrom™ and Peroxichrom Excel™ contain no organic solvents such as DMF, DMSO, methanol so there is no issue of safety with user.

Precautions:

TMB substrate is sensitive to contamination from a variety of oxidizing agents. Avoid prolonged exposure to light, contact with metal or air. we recommend using TMB substrate by pouring out required amount into a reservoir and do not return excess TMB to provided bottle.

Storage :

Stable at 4℃ or Room temperature for 1 year

Procedure:

1. Warm to room temperature prior to use if you store TMB solution at 4℃
2. Dispense 1-Component appropriate TMB solution or 100ul into each well
3. After sufficient color development (5-10minutes at room temperature or at 37℃) add 50ul Stop Solution (2N H_2SO_4 or 1M H_3PO_4) to each well.
4. Read plates at 450nm