Wako

Life Science

Pre-cast Gels for Protein Phosphorylation Research

SuperSep Phos-tag™

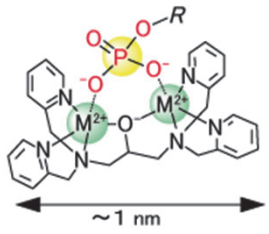
SuperSep Phos-tag™ offers a method for protein phosphorylation research without either phospho-specific antibodies or radioactive isotope labeling.

SuperSep Phos-tag™

SuperSep Phos-tag™ which are added with 50 µmol/L of Phos-tag™ and zinc ions in advance are pre-cast gels,. Phos-tag™ with zinc ions in the gels decreases the migration speed in SDS-PAGE and phosphorylated/non-phosphorylated proteins are separated as the different bands.
After separation, the gel can be utilized for CBB staining, western blotting and mass spectrometry.

Principle of Phos-tag™ SDS-PAGE

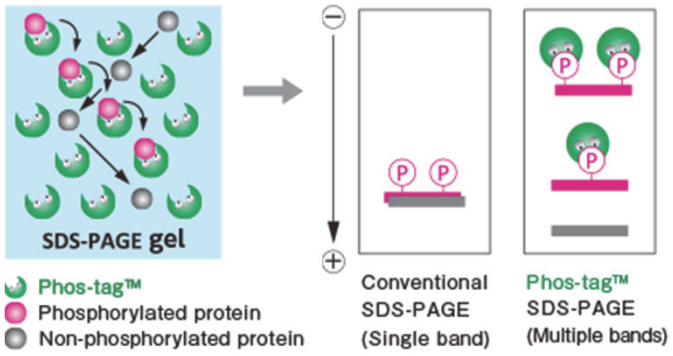
Phos-tag™ with two metallic ions cooperate to bind a phosphate group



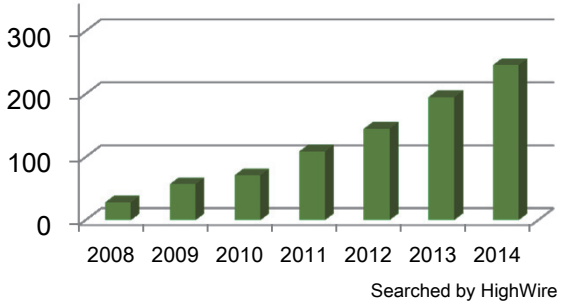
M^{2+} : Zinc ion or manganese ion

Phosphorylated proteins move while being bound by Phos-tag™ in the gel.

Migration speed of phosphorylated proteins decreases.



Number of publications citing the use of the Phos-tag™ SDS-PAGE method since 2008.

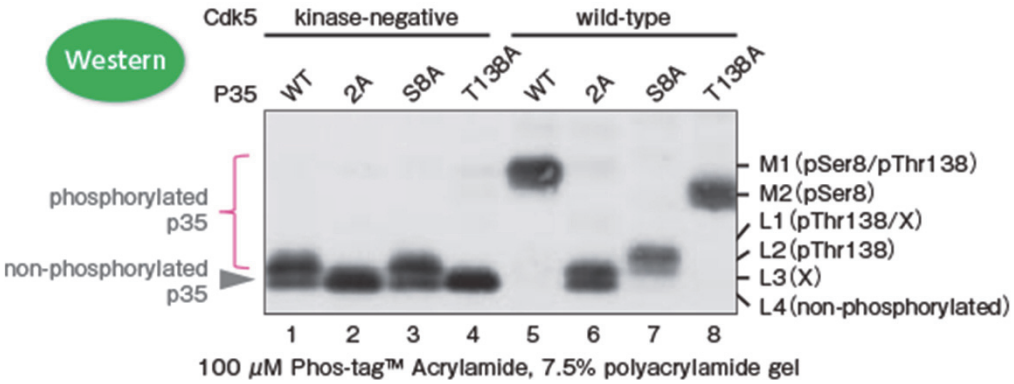


Searched by HighWire

Applications

Search for phosphorylation site of Cdk5(cyclin-dependent kinase 5)-activated sub-unit p35 using Ala substitution variant

Regarding p35 known phosphorylation sites Ser8 and Thr138, 3 Ala substitution variants were produced (Ser8: S8A, Thr138: T138A, Ser8 and Thr138：2A). These and wild-type p35, as well as Cdk5 or kinase-negative Cdk5, which has no kinase activity, were discovered in the COS-7 cells. The cellular extract was detected by Western blotting using Phos-tag™ SDS-PAGE. (Detected extract: anti-p35 antibody)



Relationship between phosphorylation site and band shift was clarified.

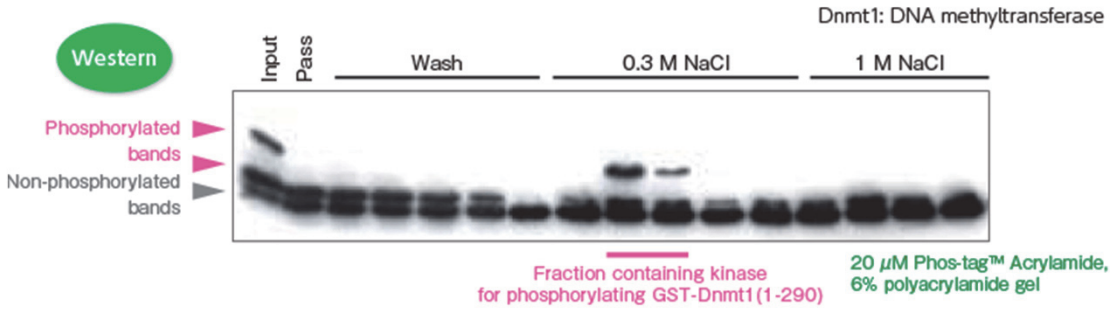
- From lanes 1 (L2, L4) and 5 (M1): p35 is phosphorylated, depending on Cdk5.
- From lanes 1 (L2, L4) and 3 (L2, L4)：With about half of p35, Thr138 is phosphorylated at kinase-negative Cdk5, and Thr138 is also phosphorylated by kinase other than Cdk5.
- From lanes 5 (M1) and 6 (L3, L4)：Ser8 and Thr138 are main phosphorylation sites.
- From lanes 5 (M1), 7 (L1, L2) and 8 (M2)：M1 is the phosphorylation site for Ser8 and Thr138. M2 is the phosphorylation site for Ser8 only. L1 and L2 are the phosphorylation sites for Thr138 only.

※X in L1, L3： not yet identified
※L4: non-phosphorylated p35

Data published in：
Quantitative Measurement of in Vivo Phosphorylation States of Cdk5 Activator p35 by Phos-tag™ SDS-PAGE. T. Hosokawa, T. Saito, A. Asada, K. Fukunaga, and S. Hisanaga, Mol. Cell. Proteomics, Jun 2010; 9: 1133 - 1143.

Data provided by：
Dr. T. Hosokawa, Laboratory for Memory Mechanisms Neural Circuit Function Research Core, Brain Science Institute, RIKEN and Dr. S. Hisanaga, Molecular Neuroscience Laboratory, Department of Biological Sciences, Graduate School of Science and Engineering, Tokyo Metropolitan University.

Determining fraction containing kinase for phosphorylating Dnmt1



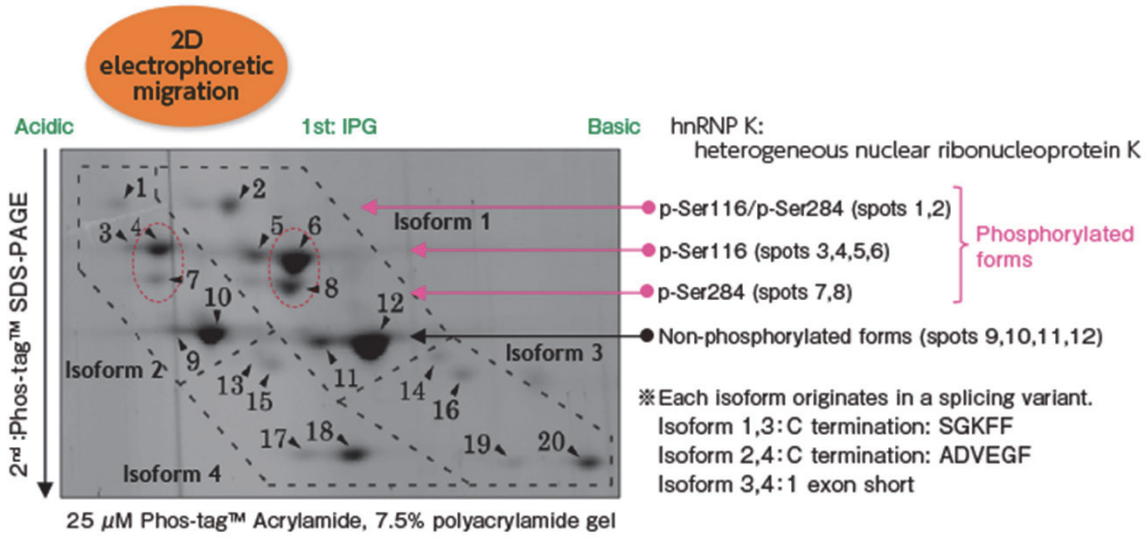
We were able to determine the fraction that contained the target kinase.

① GST-Dnmt1(1-290) bonding protein was obtained from mouse brain extract using affinity chromatography.
② Proteins were eluted through the DNA cellulose column by 0.3 M and 1 M NaCl.
③ *In vitro* kinase assay was performed in each fraction with GST-Dnmt1(1-290) as substrate.
④ Kinase activity in the fraction was confirmed by shift band, by Western blotting using Phos-tag™ SDS-PAGE (Detection: Anti mouse Dmnt1(72-86)).

Data published in:
The DNA-binding activity of mouse DNA methyltransferase 1 is regulated by phosphorylation with casein kinase 1δ/ε, Y. Sugiyama, N. Hatano, N. Sueyoshi, I. Suetake, S. Tajima, E. Kinoshita, E. Kinoshita-Kikuta, T. Koike, and I. Kameshita, *Biochem. J.*, May 2010; **427**(3): 489-97.
Data provided by:
Dr. Y. Sugiyama, Laboratory of Molecular Biology, Science Research Center, Kochi University and Dr. I. Kameshita, Department of Life Science, Faculty of Agriculture, Kagawa University.

Application in two-dimensional electrophoretic migration: Analysis of phosphorylated forms of hnRNPK

hnRNP K was isolated by immunoprecipitation from nuclear homogenate of mouse macrophage cell line J774.1 cells stimulated with LPS, and hnRNP K isoforms were separated using IPG strip gel (pH 4.7—5.9) in the first dimension and Phos-tag™ SDS-PAGE in the second dimension. Each isoform and modification site was then identified using mass spectrometry.



Each phosphorylated form was distinguished at the same isoelectric point, respectively(e.g. spots 6 vs. 8 and spots 4 vs. 7).

Data published in:
Characterization of multiple alternative forms of heterogeneous nuclear ribonucleoprotein K by phosphate-affinity electrophoresis. Y. Kimura, K. Nagata, N Suzuki, R. Yokoyama, Y. Yamanaka, H. Kitamura, H. Hirano, and O. Ohara, *Proteomics*, Nov 2010; **10**(21): 3884-95.
Data provided by:
Dr. Y. Kimura and Dr. H. Hirano, Yokohama City University and O. Ohara, RCAI, RIKEN.

Notes

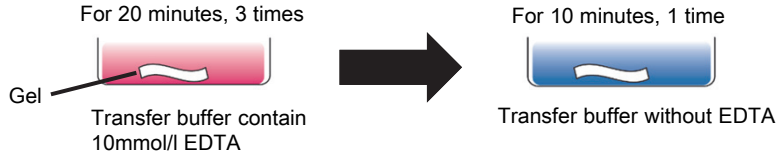
-Sampel preparation

Phos-tag SDS-PAGE is vulnerable to contaminant in protein samples, especially to chelating reagent, vanadic acid, inorganic salts, surfactants. Cleaning them up by TCA precipitation, dialysis or desalting is strongly recommended before Phos-tag SDS-PAGE.

-Pre-treatment for transfer

An additional procedure, elimination of zinc ions (Zn2+) from the gel using EDTA, is necessary before transfer. This procedure increases transfer efficiency of proteins from a gel to a membrane.

1) Prepare 1x transfer buffer with 10 mmol/L EDTA and without EDTA.
2) Soak the gel in 1x transfer buffer with 10 mmol/L EDTA for a minimum of 20 minutes with gentle agitation. Repeat it 3 times with buffer exchanges.
3) Soak the in 1x transfer buffer without 10 mmol/L EDTA for 10 minutes with gentle agitation.
4) Transfer the proteins from the gel to a membrane*.
   * A Wet-tank method is strongly recommended for effective protein transfer.



Quality Control

Every batch of SuperSep Phos-tag™ is tested for compliance with its product specification. It is checked that certain phosphorylated and dephosphorylated proteins are separable and the degrees of the proteins' mobility are constant in a practice test.

Product List

For Bio-Rad's electrophoresis tank

Code No.	Discpription	Electrophoresis Tank	Size
198-17981	SuperSep™ Phos-tag™ (50μmol/l), 7.5%, 17well, 83×100×3.9mm	Mini-PROTEAN® Tetra Cell (Bio-Rad Laboratories, Inc.)	5 Gels
195-17991	SuperSep™ Phos-tag™ (50μmol/l), 12.5%, 17well, 83×100×3.9mm		5 Gels

Mini-PROTEAN is a registered trademark of Bio-Rad Laboratories, Inc.

For Life Technologies' electrophoresis tank

Code No.	Discpription	Electrophoresis Tank	Size
192-18001	SuperSep™ Phos-tag™ (50μmol/l), 7.5%, 17well, 100×100×6.6mm	XCell SureLock® Mini-Cell (Life Technologies, Inc.)	5 Gels
199-18011	SuperSep™ Phos-tag™ (50μmol/l), 12.5%, 17well, 100×100×6.6mm		5 Gels

XCell SureLock is a registered trademark of Life Technologies, Inc.

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