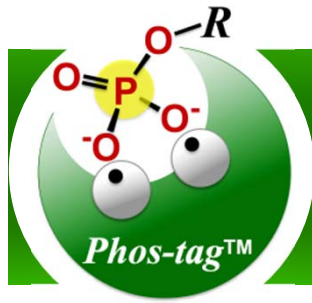


Wako



Phos-tag™ Series

★ *New Tools for Research on Phosphorylated Proteins*

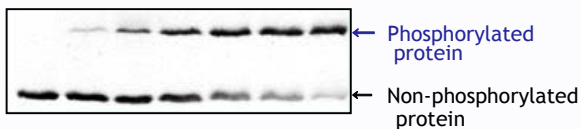
What is Phos-tag ?

Phos-tag™ is a functional molecule that specifically binds to proteins phosphorylated at Ser/Thr/Tyr and His/Asp/Lys. It is applicable to the specific separation of phosphorylated proteins as well as to Western blot detection, purification by agarose gel chromatography and MALDI-TOF/MS analysis. The following five kinds of lineup are available:

Separation

Specific separation of phosphorylated proteins

1 Phos-tag™ Acrylamide



2 SuperSep Phos-tag™ - Ready-to-use Precast Gels

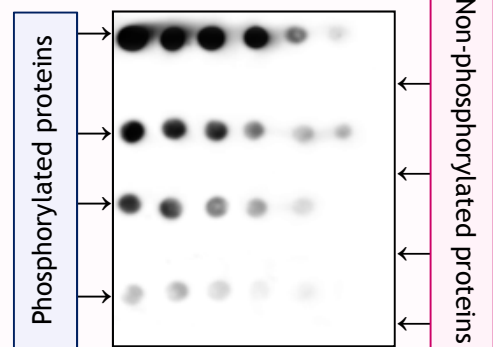


Detection

3 Phos-tag™ Biotin

Achieves specific detection without anti-phospho antibodies

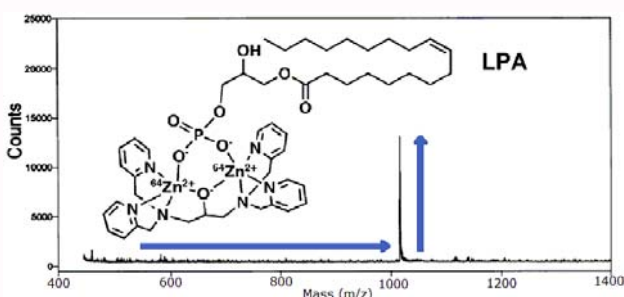
Applicable to chemiluminescence detection of phosphorylated proteins



Analysis

4 Phos-tag™ Mass Analytical Kit for MALDI-TOF MS

for high sensitive detection of phosphorylated molecules



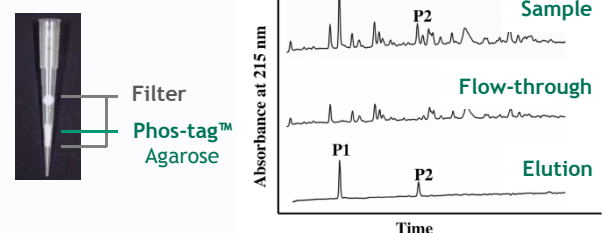
Purification

5 Assay at a physiological condition (pH 7.5) Phos-tag™ Agarose



Ready-to-use phosphorylated peptide purification tip

Phos-tag™ Tip



Phos-tag™ was developed by the Department of Functional Molecular Science at Hiroshima University.

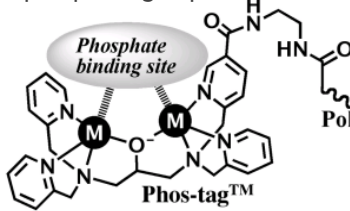
1 for the preparation of self-made acrylamide Gel Phos-tag™ Acrylamide

■ Features

1. Recognizes and separates all proteins phosphorylated at Ser/Thr/Tyr and His/Asp/Lys.
2. Corresponds to the degree and the position of phosphorylation
3. Almost the same procedure as that of a conventional SDS-PAGE
4. Operates with your electrophoresis tank

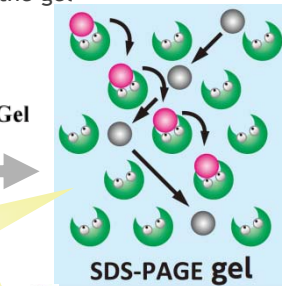
■ Principle

Two metallic ions cooperate to bind a phosphate group

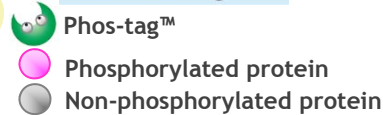


Polyacrylamide Gel

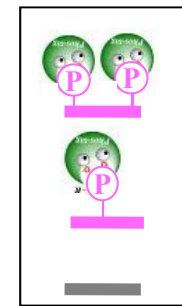
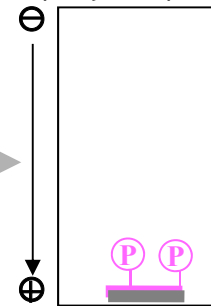
Phosphorylated proteins move while being bound by Phos-tag™ in the gel



Isoforms with different numbers and positions of phosphorylation can also be separated.



Migration speed of phosphorylated proteins decreases and they are separated from non-phosphorylated proteins.



Phos-tag™ SDS-PAGE GUIDEBOOK for researchers who use Phos-tag™ Acrylamide for the first time. Please contact us.



2 Ready-to-use precast gels SuperSep Phos-tag™ (50 μmol/L Phos-tag™ Acrylamide)

■ Features

1. Ready-to-use
2. Safety due to precast gel
3. Long-term stability due to neutral gel buffer (Stable for 9 months)
4. Almost the same basic mechanism as that of SDS-PAGE
5. High reproducibility

Note:

Before using SuperSep precast gel, check the sample for migration pattern with conventional SDS-PAGE.

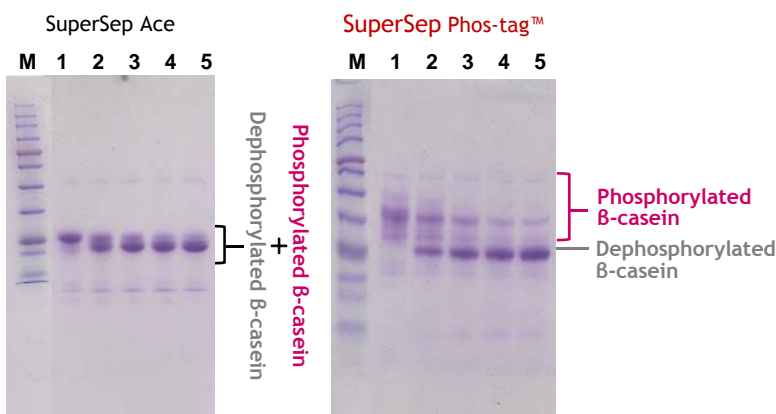
Electrophoresis tank	Mini-PROTEAN® Tetra Cell (Bio-Rad Laboratories, Inc.) or XCell SureLock® Mini-Cell (Life Technologies, Inc.)	Number of wells	17
		Phos-tag™ Conc.	50 μmol/L
		Acrylamide Conc.	7.5% or 12.5%
		ZnCl ₂ Conc.	100 μmol/L
		Maximum well volume	25 μL

Mini-PROTEAN is a registered trademark of Bio-Rad Laboratories, Inc.
XCell SureLock is a registered trademark of Life Technologies, Inc.



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■ Application ~ Dephosphorylation time course of β-casein ~



[Buffer] Tris-Glycine-SDS buffer
[Sample] M: WIDE-VIEW™ Prestained Protein Size Marker III
1: 0 min. β-casein
2: 15 min. β-casein
3: 30 min. β-casein
4: 45 min. β-casein
5: 60 min. β-casein
Alkaline phosphatase treated

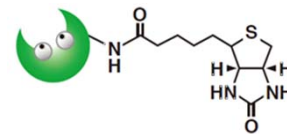
[Condition] Constant current 35 mA for 60 min.
[Staining] Quick-CBB Staining (Wako Cat. No. 299-50101)
[Destaining] Deionized water with microwave treatment

β-casein was dephosphorylated over time.
Dephosphorylated β-casein can be separated from β-casein with SuperSep Phos-tag™.

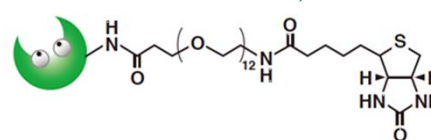
3 for specific detection of phosphoproteins on PVDF membrane Phos-tag™ Biotin

This biotin-bound Phos-tag™ is used for the detection of phosphoproteins by Western blotting. Specific detection without any anti-phospho antibodies on Western blot. Detection is possible regardless of the type of phosphorylated amino acid. BTL-104 and BTL-111 have linkers with different lengths. BTL-111 offers higher sensitivity.

<BTL-104> Mol. Wt.: 767.0



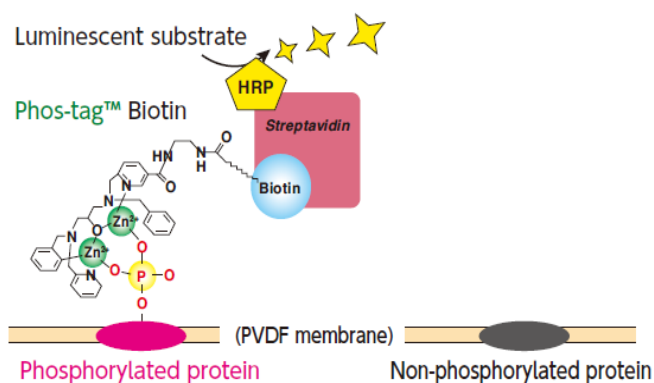
<BTL-111> Mol. Wt.: 1,367



Features

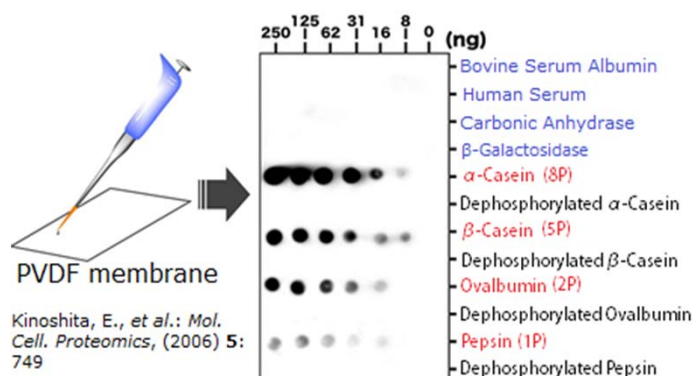
1. All phosphoproteins can be detected.
2. Procedures of the experiment are similar to those in ordinary Western blotting.
3. Specific detection without any anti-phospho antibodies on Western blot.

Schematic of Western blotting



Application: Dot-blotting analysis

- Chemiluminescence detection of phosphorylated proteins on PVDF



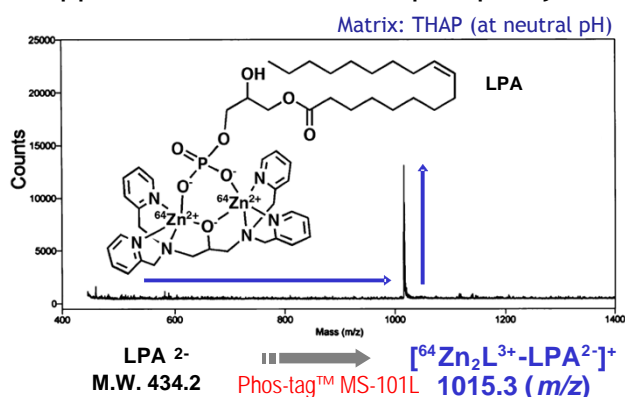
Description		Pkg. Size	Wako Cat. No.	Manufacturer (Prod. #)
For specific separation of proteins phosphorylated at Ser/Thr/Tyr				
1	Phos-tag™ Acrylamide AAL-107, 5 mM Aqueous Soln.	0.3 mL (0.9 mg)	304-93526	NARD (AAL-107S1)
	Phos-tag™ Acrylamide AAL-107	2 mg	300-93523	NARD (AAL-107M)
		10 mg	304-93521	NARD (AAL-107)
Ready-to-use Phos-tag™ precast gels for Mini-PROTEAN® Tetra Cell (Bio-Rad Laboratories, Inc.)				
2	SuperSep™ Phos-tag™ (50 μmol/L), 7.5%, 17well, 83 × 100 × 3.9 mm	5 gels	198-17981	Wako
	SuperSep™ Phos-tag™ (50 μmol/L), 12.5%, 17well, 83 × 100 × 3.9 mm	5 gels	195-17991	
Ready-to-use Phos-tag™ precast gels for XCell SureLock® Mini-Cell (Life Technologies, Inc.)				
2	SuperSep™ Phos-tag™ (50 μmol/L), 7.5%, 17well, 100 × 100 × 6.6 mm	5 gels	192-18001	Wako
	SuperSep™ Phos-tag™ (50 μmol/L), 12.5%, 17well, 100 × 100 × 6.6 mm	5 gels	199-18011	
For detection of phosphoproteins on PVDF membrane				
3	Phos-tag™ Biotin BTL-104	10 mg	301-93531	NARD (BTL-104)
	Phos-tag™ Biotin BTL-111, 1 mM Aqueous Soln.	0.1 mL	308-97201	NARD (BTL-111S1)

Mini-PROTEAN is a registered trademark of Bio-Rad Laboratories, Inc. XCell SureLock is a registered trademark of Life Technologies, Inc.

4 for MALDI-TOF/MS with high sensitivity Phos-tag™ Mass Analytical Kit

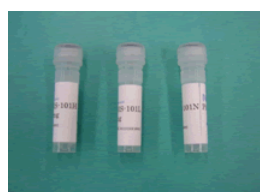
Phosphorylated molecules are usually difficult to be detected by MALDI-TOF/MS. This product enables improvement of the sensitivity and accuracy of the analysis. Before use, Phos-tag™ Mass Analytical Kit is mixed with samples for MALDI-TOF/Mass analysis. The phosphorylated molecule-Phos-tag™ complex is detected in a positive mode.

Application: Detection of phosphorylated LPA complex with Phos-tag™



【Kit Contents】

- Phos-tag™ MS-101L ($C_{27}H_{29}N_6O^{64}Zn_2^{3+}$ MW: 581.4) 5 mg
- Phos-tag™ MS-101H ($C_{27}H_{29}N_6O^{68}Zn_2^{3+}$ MW: 589.4) 5 mg
- Phos-tag™ MS-101N ($C_{27}H_{29}N_6OZn_2^{3+}$ MW: 584.3) 10 mg



Phos-tag™ Mass Analytical Kit

By the increased positive charge, detection sensitivity of phosphorylated LPA is improved

Purification



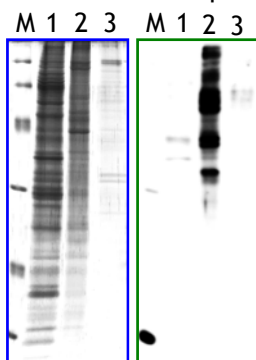
5 Purification of phosphorylated proteins by affinity chromatography Phos-tag™ Agarose

Features

1. Phosphorylated proteins can be purified within 1 hour.
2. Proteins can be trapped at physiological condition (pH 7.5).
3. Purified without reducing agents or surfactants.

Application:

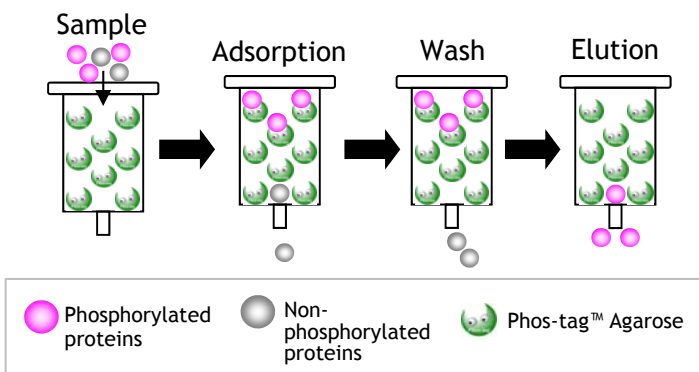
-Purification of phosphorylated proteins in A341 lysate



M: Molecular Weight Marker
Lane 1: Flow-through fraction
Lane 2: **Elution fraction**
Lane 3: Washing fraction

Fill Phos-tag™ Agarose into a column.
Apply A341 cell lysate on it.
Detection by SYPRO Ruby gel staining (left figure) and Western blotting using Anti pTyr (right figure).
Phosphorylated proteins are confirmed to be enriched in the elution fraction.

Principle of Phosphate Affinity Chromatography



	Description	Pkg. Size	Wako Cat. No.	Product No.	Note
4	Phos-tag™ Mass Analytical Kit	1 Set	305-93551	MS-101KIT	For MALDI-TOF MS
5	Phos-tag™ Agarose	0.5 mL	302-93561	AG-501	For enrichment, separation and purification using column chromatography
		3 mL	308-93563	AG-503	
	Phos-tag™ Tip	8 tips	387-07321	AG2-103	Ready-to-use tip for the concentration of phosphorylated peptides

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use.
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Online Catalog: www.e-reagent.com

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Toll-Free (U.S. only): 1-877-714-1920
Head Office (Richmond, VA):
Tel: 1-804-714-1920
Fax: 1-804-271-7791

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