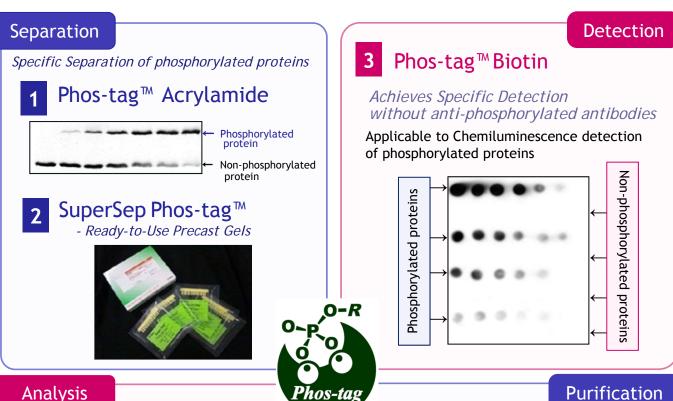


Phos-tagTM Series

★ New Tools for Research on Phosphorylated Proteins

What's Phos-tag?

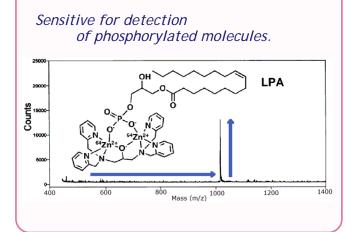
Phos-tag[™] is a functional molecule that specifically binds all phosphorylated forms of Ser/Thr/Tyr. It is applicable for the specific separation of phosphorylated proteins as well as for the western blot detection, purification by agarose gel chromatography and MALDI-TOF/MS analysis. Following five kinds of lineup are available:



Analysis

Purification by gel chromatography Phos-tag™ Agarose

M 1 2 3



Phos-tag™ Mass Analytical Kit

For MALDI-TOF MS

be trapped in physiological condition (pH 7.5). [Application] Purification of phosphorylated protein in A431 lysate M: Molecular Marker Lane 1: Non absorbed fraction Lane 2: Absorbed fraction Lane 3: Colum rinsing fraction

Phosphorylated proteins can

Separation

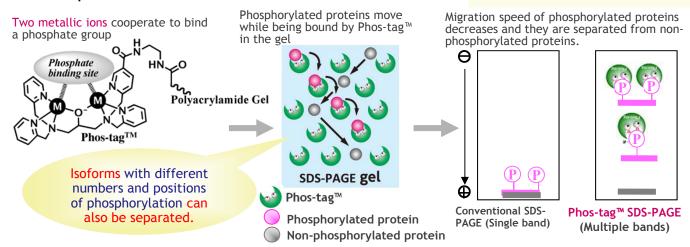




Features

- 1. Recognizes and separates all phosphorylated forms of Ser / Thr / Tyr
- 2. Corresponds to the degrees and the positions of phosphorylation
- 3. Almost the same procedure as that of a conventional SDS-PAGE
- 4. Operates with your electrophoresis tank

Principle



Ready-to-Use Precast Gels SuperSep Phos-tag TM (50 µmol/L Phos-tag TM Acrylamide contained)

Wako

Features

- 1. Ready-to-use
- 2. Safety due to precast gel
- 3. Long-term stability (Stable for 6 months)
- 4. Almost the same basic mechanism as that of SDS-PAGE
- 5. Excellent storage stability by its neutral gel buffer.

Note:

Before using SuperSep precast gel, check a sample for migration pattern problems with "SuperSep Ace" or conventional SDS-PAGE.



SuperSep Phos-tag™



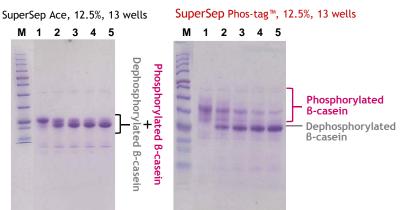
Phos-tag™ PAGE GUIDEBOOK for researchers who use Phos-tag™ Acrylamide newly. Please contact us.

EasySeparator

– an electrophoresis tank
optimized with SuperSep
precast gel

Plate Size	100 x 100 x 3 (mm)					
Gel Size	90 x 85	90 x 85 x 1 (mm) Acrylamide Conc. 10%; 1		10%; 12.5%;	; 12.5%; 15% or 17.5 %	
Well number	13	17	ZnCl ₂ Conc.	100 μmol/L		
Phos-tag™ Conc.	50 μmol/L		Well volume	30 µL	25 μL	

Application ~ Dephosphorylation over time of B-casein ~



[Buffer] Tris-Glycine-SDS electrophoresis buffer [Sample]

M: WIDE-VIEW™ Prestained Protein Size Marker III

1: 0 min. B-casein (with AP treatment)

2: 15 min. B-casein (with AP treatment)

3: 30 min. β-casein (with AP treatment) 4: 45 min. β-casein (with AP treatment)

5: 60 min. B-casein (with AP treatment)

AP: alkaline phosphatase

[Condition] Constant current 35 mA for 60 min.

[Staining] Quick-CBB Staining

(Wako Cat. No. 299-50101)

[Destaining] Deionized water with microwave treatment

B-casein was dephosphorylated over time. Dephosphorylated B-casein can be separated from B-casein with SuperSep Phos-tag $^{\text{TM}}$.



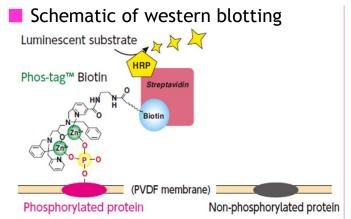


This is biotin-bound Phos-tag™ used for detection of phosphoproteins by western blotting. Specific detection without any anti-phosphorylated antibodies on Western blot. Detection is possible regardless of type of phosphorylated amino acid. BTL-104, BTL-105 and BTL-111 have linkers with different lengths. Although the usage of BTL-104 and BTL-105 are similar, BTL-104 is

BTL-104, BTL-105 and BTL-111 have linkers with different lengths. Although the usage of BTL-104 and BTL-105 are similar, BTL-104 is recommendable as the first choice because of its high solubility. BTL-111 offers higher sensitivity.

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-phosphorylated antibodies on western blot.



Application: Dot-blotting analysis - Chemiluminescence detection of phosphorylated proteins on PVDF 250 1 62 1 16 1 ° (ng) Bovine Serum Albumin Human Serum Carbonic Anhydrase β-Galactosidase a-Casein (8P) Dephosphorylated α-Casein B-Casein (5P) Dephosphorylated β -Casein PVDF membrane Ovalbumin (2P) Dephosphorylated Ovalbumin Kinoshita, E., et al.: Mol. Cell. Proteomics, (2006) 5: Pepsin (1P) 749 Dephosphorylated Pepsin

	Description	Pkg. Size	Wako Cat. No.	Manufacturer (Prod. #)	
For Sp	ecific Separation of Phosphorylated Forms of Ser/Thr/Tyr			_	
	Phos-tag™ Acrylamide AAL-107, 5 mM Aqueous Soln.	0.3 mL (0.9 mg)	304-93526	NARD (AAL-107S1)	
1	Phos-tag™ Acrylamide AAL-107	2 mg	300-93523	NARD (AAL-107M)	
	Phos-tag Actylamide AAL-107	10 mg	304-93521	NARD (AAL-107)	
Ready	-to-Use Phos-tag™ Precast Gels				
	SuperSep Phos-tag™ (50 μmol/L), 10%, 13 well	5 gels	193-16711		
2	SuperSep Phos-tag™ (50 μmol/L), 10%, 17 well	5 gels	190-16721		
	SuperSep Phos-tag™ (50 μmol/L), 12.5%, 13 well	5 gels	195-16391		
	SuperSep Phos-tag™ (50 μmol/L), 12.5%, 17 well	5 gels	193-16571		
	SuperSep Phos-tag™ (50 μmol/L), 15%, 13 well	5 gels	193-16691	Wako	
	SuperSep Phos-tag™ (50 μmol/L), 15%, 17 well	5 gels	196-16701		
	SuperSep Phos-tag™ (50 μmol/L), 17.5%, 13 well	5 gels	197-16851		
	SuperSep Phos-tag™ (50 μmol/L), 17.5%, 17 well	5 gels	194-16861		
Relate	ed Products				
	EasySeparator <an electrophoresis="" for="" supersep="" tank=""></an>	1 set	058-07681	Wako	
ľ	Wide-View Prestained Protein Size Marker III (11-245 kDa)	500 μL (200 tests)	230-02461		
For De	etection of Phosphoproteins on PVDF membrane				
	Phos-tag™ Biotin BTL-104	10 mg	301-93531	NARD (BTL-104)	
3	Phos-tag™ Biotin BTL-105	10 mg	308-93541	NARD (BTL-105)	
	Phos-tag™ Biotin BTL-111, 1 mM Aqueous Soln.	0.1 mL	308-97201	NARD (BTL-111S1)	

Analysis



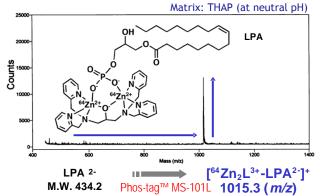
MALDI-TOF/MS with High Sensitivity

Phos-tag™ Mass Analytical Kit



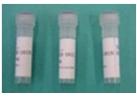
Before use, Phos-tag™ Mass Analytical Kit is mixed with samples for MALDI-TOF/Mass analysis. Phosphorylated molecule-Phos-tag™ complex is detected in a positive mode, and phosphorylated molecule usually difficult to detect can be detected with improved sensitivity.

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



(Kit Contents)

- Phos-tag[™] MS-101L (C₂₇H₂₆N₆O⁶⁴Zn₂³⁺ MW: 581.4)
 5 mg
- Phos-tagTM MS-101H $(C_{27}H_{29}N_6O^{68}Zn_2^{3+} MW: 589.4)$ ■ 5 mg
- Phos-tag[™] MS-101N (C₂₇H₂₉N₆OZn₂³⁺ MW: 584.3) · · · 10 mg



Phos-tag™ Mass Analytical Kit

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

Purification



Purification of Phosphorylated proteins by Affinity Chromatography Phos-tag™ Agarose



Features

- 1. Phosphorylated proteins can be purified in 1 hour.
- 2. Proteins can be trapped in physiological condition (pH 7.5).
- 3. Purified with no reducing agent or surfactant.

Application:

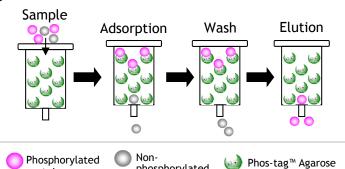
-Purification of Phosphorylated proteins in A341 lysate

M123

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

Place Phos-tag™ Agarose into a column. Apply A431 cell lysate on it. By detections of SYPRO Ruby gel staining (left figure) and western blotting using Anti pTyr (right figure), phosphorylated proteins were confirmed to enrich in the elution

Principle of Phosphate Affinity Chromatography



phosphorylated

	Description	Pkg. Size	Wako Catalog No.	Product No.	Note
	Phos-tag™ Mass Analytical Kit	1 Set	305-93551	MS-101KIT	For MALDI-TOF MS
	Phos-tag™ Agarose	0.5 mL	302-93561	AG-501	For enrichment, separation and
	Thos tag Agarose	3 mL	308-93563	AG-503	purification

proteins

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. Please visit our online catalog to search for other products from Wako: http://www.e-reagent.com

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Tel: 81-6-6203-3741 Fax: 81-6-6203-1999

Online Catalog: www.e-reagent.com

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www.wakousa.com

Toll-Free (U.S. only): 1-877-714-1920

Head Office (Richmond, VA): Tel: 1-804-714-1920/Fax: 1-804-271-7791 Los Angeles Sales Office (Irvine, CA):

Tel: 1-949-679-1700/Fax: 1-949-679-1701 Boston Sales Office (Cambridge, MA): Tel: 1-617-354-6772/Fax: 1-617-354-6774

Wako Chemicals GmbH

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