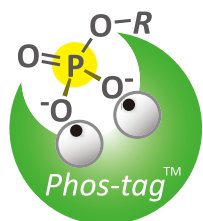


# Wako

## Phos-tag™ SDS-PAGE GUIDEBOOK



# Phos-tag™

[phos-tag.jp](http://phos-tag.jp)

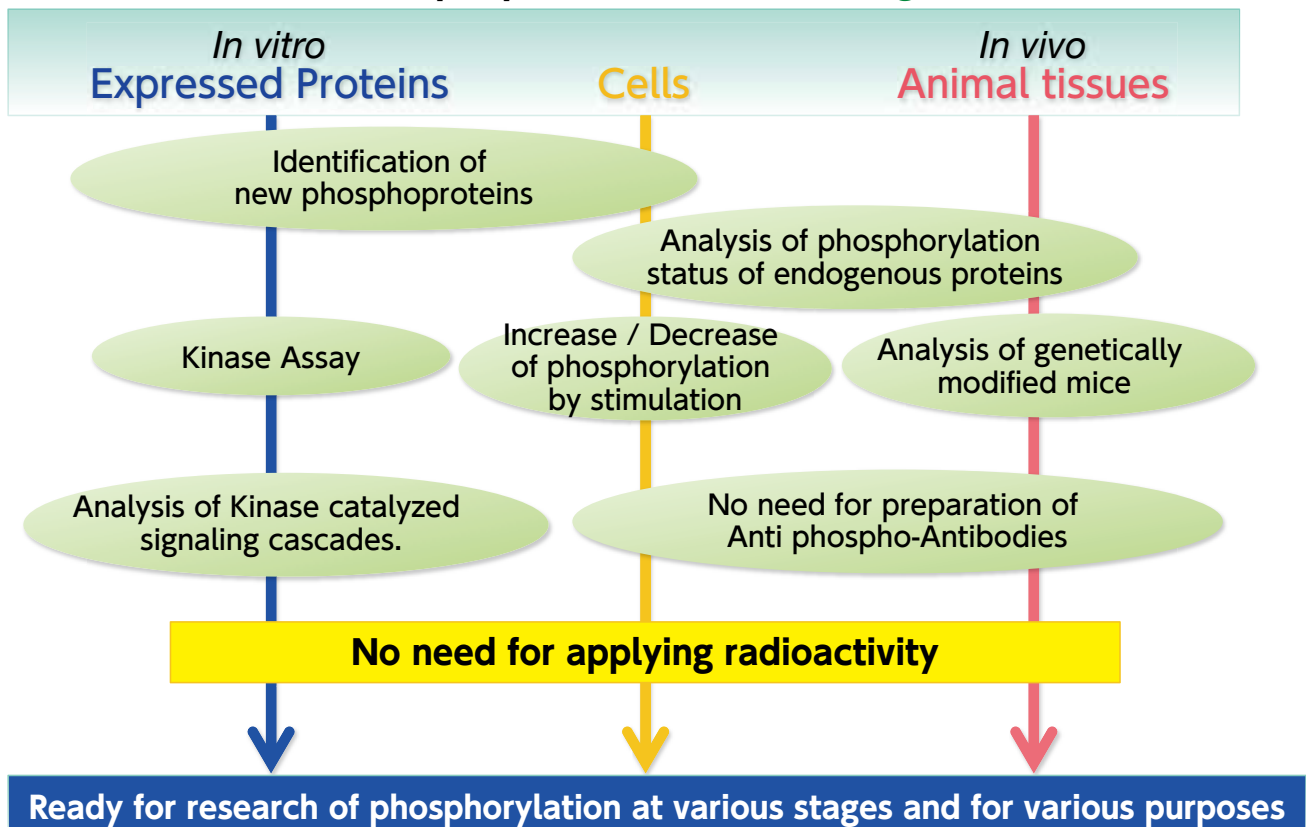
**Wako**

# INDEX

1. Principle and Application	P3
2. Phos-tag™ SDS-PAGE is...	P4
3. Protocol	P6
[I] Mn <sup>2+</sup> -Phos-tag™ SDS-PAGE	P6
[II] Zn <sup>2+</sup> -Phos-tag™ SDS-PAGE	P11
4. Trouble Shooting	P14
5. Optimization of Phos-tag™ SDS-PAGE Condition	P17
6. Application Data and References	P18
7. FAQ (Phos-tag™ Series)	P22
8. SuperSep Phos-tag™	P26
9. Phos-tag™ Series	P27
10. Related Products (Electrophoresis/Western Blotting)	P29

## Phos-tag™ Applications

### Multiple possibilities of Phos-tag™

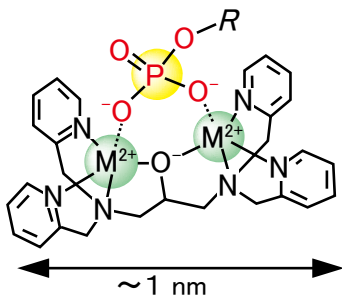


# 1. Principle and Application

## Phos-tag™ is ...

Phos-tag™ is a functional molecule which captures phosphorylated Ser/Thr/Tyr and His/Asp/Lys. It is a synthesized chemical compound designed from an alkaline phosphatase catalytic domain as a model. A series of reagents utilizing Phos-tag™ can be used in the separation, detection, mass spectrometry analysis, and purification of phosphorylated proteins.

### [Basic Structure of Phos-tag™]



M<sup>2+</sup> : Zinc ion or manganese ion

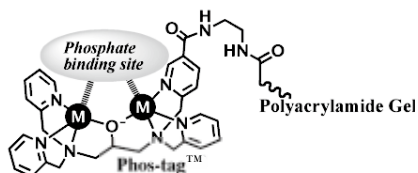
- ◇ Selectivity of binding of a phosphate ion (2-) is much higher than that of other anions.
- ◇ Stable complex is formed under physiological conditions (pH 5 to 8).

Product	Purpose of Use
Phos-tag™ Acrylamide	Separation : Separation is possible by SDS-PAGE depending on the degree of phosphorylation.
SuperSep Phos-tag™	Separation : Ready-to-use precast gel containing 50 μM Phos-tag™ Acrylamide
Phos-tag™ Biotin	Detection : A substitute for the anti-phospho antibody used in western blot.
Phos-tag™ Agarose	Purification : Phosphorylated proteins are purified by column chromatography.
Phos-tag™ Tip	Purification : Ready-to-use tip to purify phosphorylated peptides
Phos-tag™ Mass Analytical Kit	Analysis : This is used in MALDI-TOF/MS analysis to improve the detection sensitivity of phosphorylated molecules.

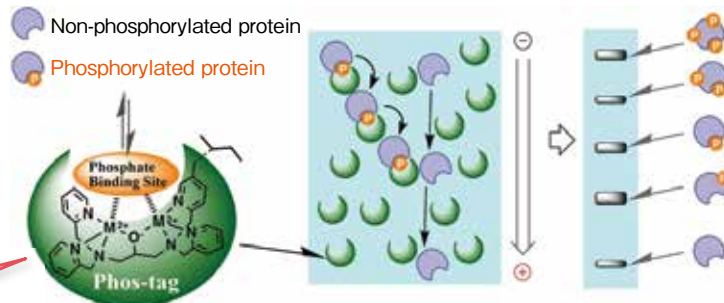
Phos-tag™ was developed by the Department of Functional Molecular Science at Hiroshima University. <http://www.phos-tag.com/>

## Principle

Two metallic ions cooperate to bind a phosphate group



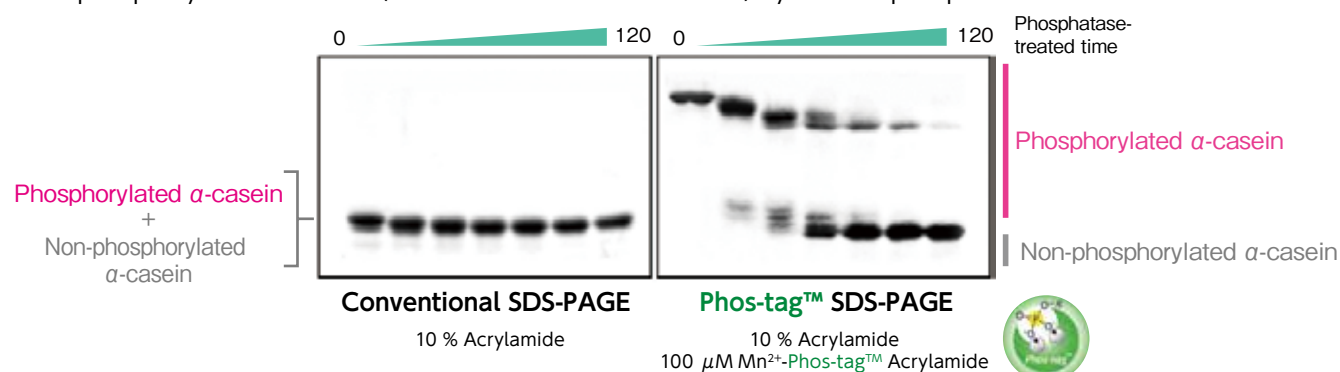
Phosphorylated forms can be separated by the amount and site of phosphorylation.



1. Divalent metal ions trap phosphorylated proteins during migration.
2. The higher the amount of phosphorylation, the slower the migration velocity.
3. Separation occurs based on phosphorylation levels. (Separation can occur if the phosphorylation sites are different, even with identical levels of phosphorylation)

## Application — Time-course of α-casein dephosphorylation —

Phos-tag™ SDS-PAGE and conventional SDS-PAGE was used to separate α-casein samples, which have been dephosphorylated over time (incubation duration: 0–120 min) by alkaline phosphatase.



## 2. Phos-tag™ SDS-PAGE is ...

### ■ Phos-tag™ SDS-PAGE

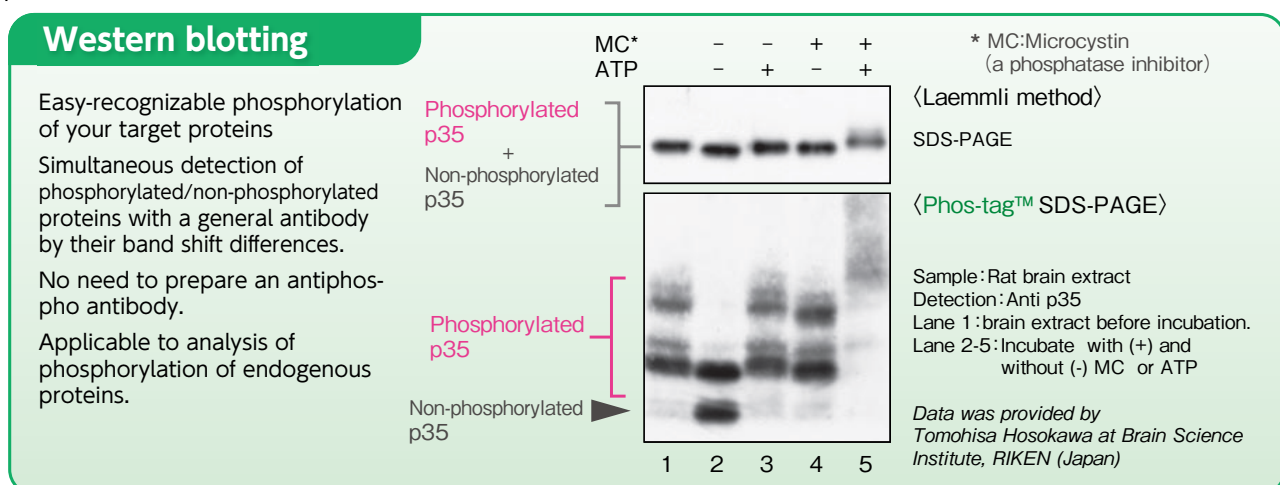
Phos-tag™ SDS-PAGE is an electrophoresis technique capable of separating phosphorylated and non-phosphorylated forms based on phosphorylation levels. Various stains, Western blotting (WB) and mass spectrometry (MS) can be employed after electrophoresis. Phos-tag™ SDS-PAGE gels can be produced by adding a divalent metal ( $MnCl_2$  or  $ZnCl_2$ ) and Phos-tag™ Acrylamide (Phos-tag™ molecule bound to acrylamide) to the SDS-PAGE resolving gel.

### ■ Features

- ◆ **It can be used regardless of the type and position of amino acid residues.**
  - It can be used in phosphorylation analysis of unknown phosphoproteins.
  - It can be used in the detection of new phosphorylation sites.
- ◆ **Phosphorylated forms with different amounts and location of phosphorylation sites can be separated.**
  - Able to determine the level of phosphorylation, as well as the amount of phosphorylated forms.
- ◆ **Capable of simultaneously detecting phosphorylated and non-phosphorylated forms.**
  - Able to quantify various phosphorylated forms.
  - Easily determine the presence of phosphorylation
- ◆ **Radioisotopes or special equipment is not necessary (can be used immediately if there are SDS-PAGE reagents and equipment)**
  - Experiments can be carried out easily and at low costs.
- ◆ **After electrophoresis, WB- and MS-based analysis, as well as 2D gel electrophoresis can be performed.**
  - **WB** : internal control protein analysis is possible. (See Application Data 3 and 4 on page #20.)
  - **MS** : phosphorylation site combinations of various phosphorylated forms can be determined. (See Application Data 1 and 2 on page #19.)
  - **2D gel electrophoresis** : phosphorylated forms with identical isoelectric points or molecular weights can be separated. (See Application Data 2 on page #19.)

### ■ Development from Phos-tag™ SDS-PAGE

By the combination of Phos-tag™ SDS-PAGE with various analysis methods, new information of phosphorylated proteins can be obtained.



### Mass Analysis

By separating phosphorylated forms, each phosphorylation site combination can be detected.

### 2D Electrophoresis

Phosphorylated forms with the same isoelectric point (same number of phosphorylation sites) can be separated.  
Application Data → See the page #18

Objective	Sample type	Application	Related Products	Application Data
To identify phosphorylation sites	Lysate	Ala substitute + WB	ImmunoStar Series	p.19 ④
	Purified protein	Recombinant + MS Immunoprecipitation + MS	Silver Stain MS Kit, nanoLC-MS/MS	p.18 ①, ②
To simultaneously detect and quantify various phosphorylated forms	Lysate	WB	ImmunoStar Series	p.19 ④, p.20 ⑤, ⑥
	Purified protein	CBB staining, Silver staining, etc.	Quick CBB Plus, Silver Stain MS Kit, etc.	p.18 ①, ②
To confirm presence of phosphorylation	Lysate	WB	ImmunoStar Series	p.19 ③, p.20 ⑤, ⑥
To perform additional separation	Immunoprecipitation sample	2D Electrophoresis	Quick CBB Plus, Silver Stain MS Kit, etc.	p.18 ②
To search for kinase or inhibitors effective against the target protein	Lysate	Column Chromatography + WB	ImmunoStar Series	p.19 ③
	Purified protein	Recombinant kinase + CBB staining, Silver staining, etc.	-	p.18 ①

Product Name	Pkg. Size	Wako Cat. No.(Nard Product #)	Note
Phos-tag™ Acrylamide 5 mM Aqueous Solution	0.3 mL (0.9 mg)	304-93526(AAL-107S1)	Prepared aqueous solution
Phos-tag™ Acrylamide	2 mg	300-93523(AAL-107M)	Prepare with methanol or water
	10 mg	304-93521(AAL-107)	

### Important differences compared to conventional SDS-PAGE and cautions

There are some important differences between Phos-tag™ SDS-PAGE and conventional SDS-PAGE that should be considered.

#### ◆ Prior sample preparation is required.

Due to Phos-tag™ SDS-PAGE being easily influenced by EDTA, prior sample processing (such as TCA precipitation) is highly recommended ⇒ [Details on page #14](#).

#### ◆ Migration velocity is slower

Even with non-phosphorylated proteins, the migration velocity in Phos-tag™ SDS-PAGE is lower than in conventional SDS-PAGE. ⇒ [Details on page #17](#).

#### ◆ Unable to perform molecular weight estimations using molecular weight markers.

In Phos-tag™ SDS-PAGE, molecular weight estimations using molecular weight markers is not possible. Please simply use the markers as a guide of WB transfer efficiency. ⇒ [Details on page #23](#). Additionally, pre-stained markers can cause band distortions. It is recommended to use a recombinant or dephosphorylated sample of the target protein as a marker. ⇒ [Details on page #14](#).

#### ◆ EDTA treatment is required for WB transfer.

In order to increase the transfer efficiency of Phos-tag™ SDS-PAGE to WB, EDTA treatment is required prior to transfer.

#### ◆ When performing Phos-tag™ SDS-PAGE, please concurrently perform a conventional SDS-PAGE as a control.

This is required when multiple bands are detected in Phos-tag™ SDS-PAGE, in order to determine whether the target protein is phosphorylated or degraded.

# 3. Protocol

## Two Phos-tag™ SDS-PAGEs

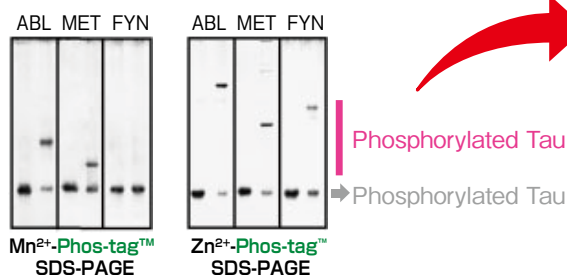
Depending on the type of divalent metal ion that binds to the Phos-tag™ molecule and the composition of buffers used in gel preparation, Phos-tag™ SDS-PAGE gels can be separated into two types as shown below. Since there are various features available, please choose accordingly to your needs. Various examples of usage are described on page #17.

[ I ] **Mn<sup>2+</sup>-Phos-tag™ SDS-PAGE Gel** [1] Composition based on the Laemmli method ⇒ p.18-20 ②③④⑤

**NEW!**

[ II ] **Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE Gel** [2] Composed of a neutral buffer ⇒ p.18 ①

Gel Type	Advantages	Disadvantages
<b>Mn<sup>2+</sup>-Phos-tag™ SDS-PAGE</b>	<ul style="list-style-type: none"> <li>• Protocol similar to Laemmli method</li> <li>• Semi-dry transfer possible even with high concentrations of Phos-tag™</li> </ul>	<ul style="list-style-type: none"> <li>• Some proteins are inseparable.</li> <li>• Gel preparation should be just before use.</li> </ul>
<b>Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE</b>	<ul style="list-style-type: none"> <li>• High resolving capability</li> <li>• Ability to separate a wide range of proteins and phosphorylated forms</li> <li>• Long storage life of gels</li> </ul>	<ul style="list-style-type: none"> <li>• Semi-dry transfer rate is poor at high concentrations of Phos-tag™</li> <li>• Bands will smear in low concentration gels with less than 5%.</li> </ul>



80μM Phos-tag™ Acrylamide, 7.5 % Polyacrylamide gel  
Detection : Fluorescent staining

### In Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE

- Resolving capabilities were improved. (ABL, MET)
- Bands unable to be separated on Mn<sup>2+</sup>-Phos-tag™ SDS-PAGE could be resolved. (FYN)

[Sample of each lane]  
Left : non-phosphorylated Tau  
Right : phosphorylated Tau  
(phosphorylated form by ABL, MET, FYN)

Improved Phos-tag SDS-PAGE under neutral pH conditions for advanced protein phosphorylation profiling.  
E Kinoshita and E Kinoshita-Kikuta, *Proteomics*, Jan 2011; 11(2): 319-23.

## [ I ] Mn<sup>2+</sup>-Phos-tag™ SDS-PAGE

Note: Always prepare the gel just before use

### ① Preparation of reagents for Phos-tag™ SDS-PAGE

Acrylamide Solution

: The ready-to-use solution is available. Please refer to 10. Related Products.

**Sol. A : 30 w/v% Acrylamide/Bis Mixed Solution (30% T, 3.3% C)**

Acrylamide ..... 29.0 g  
N, N'-methylene-bisacrylamide ..... 1.0 g

⇒ Prepare the 100 mL solution by adding distilled water and filter the solution.  
[Storage] Keep at 4°C in the dark.

Wako Catalog No. 015-25635  
30 w/v% Acrylamide/Bis Mixed Solution, 29:1 (500 mL)



Tris-HCl Buffer for Resolving Gel, pH 8.8

**Sol. B : 1.5 mol/L Tris/HCl Solution, pH 8.8 (4x solution for Resolving Gel)**

# Tris base  
(MW : 121, pKa= 8.2 at 20°C) ..... 18.2 g  
# 6.0 mol/L HCl  
(0.19 equivalents of Tris) ..... 4.85 mL

⇒ Prepare the 100 mL solution by adding distilled water and filter the solution.  
[Storage] Keep at 4°C in the dark.

Wako Catalog No. 192-11041  
Separating Gel Buffer Soln. (x4) (250 mL) containing SDS.



Tris-HCl Buffer  
for Stacking Gel,  
pH 6.8

**Sol. C : 0.50 mol/L Tris/HCl Solution, pH 6.8 (4x solution for Stacking Gel)**

- # Tris base ..... 6.06 g  
# 6.0 mol/L HCl  
(0.96 equivalent of Tris base) ..... 8.0 mL  
# distilled water ..... 90 mL

Wako Catalog No.199-11051  
Stacking Gel Buffer Soln. (x4),  
containing SDS (250 mL)



⇒ Adjust the pH to 6.8 using 6.0 mol/L HCl (ca. 0.1 mL), then prepare the 100 mL solution by adding distilled water.

**[Storage]** Keep at 4°C

SDS Solution

**Sol. D : 10% (w/v) SDS Solution**

- # SDS ..... 10.0 g  
# distilled water ..... 90 mL

Wako Catalog No. 311-90271  
10% SDS Soln. (100 mL)

Wako Catalog No. 313-90275  
10% SDS Soln. (500 mL)  
Manufactured by Nippon Gene



⇒ After stirring, prepare the 100 mL solution by adding distilled water

**[Storage]** Keep at 4°C.

Phos-tag™ AAL Soln.  
Ready-to-Use Soln.  
is available. Please  
see the page #4  
(Wako Cat. No. 304-  
93525)

**Sol. E : 5.0 mmol/L Phos-tag™ Solution containing 3% (v/v) methanol**

※The amount shown in parentheses are required  
for 2 mg of Phos-tag™ AAL-107

- # Phos-tag™ AAL-107 (MW: 595) ..... 10 mg(2 mg)  
# methanol ..... 0.10 mL(0.02 mL)  
# distilled water ..... 3.2 mL(0.64 mL)

**NARD** Institute, Ltd.



Phos-tag™ Acrylamide AAL-107

The oily product, Phos-tag™ AAL-107 is provided  
in a small plastic tube and has to be completely  
dissolved in 0.1 mL methanol.



The methanol solution should be diluted  
with 3.2 mL of distilled water by pipetting.

Note: If a trace amount of insoluble material appears as white fine powder  
(impurity) in the solution, it can be separated by centrifuging  
(2000 x g, 10 min) using 2-mL microtubes.



Just after adding water into  
Phos-tag™ MeOH soln.

**[Storage]** Wrap the tube with aluminum foil.

Keep the soln. in a 2-mL microtube at 4°C in the dark.

Phos-tag™ Aqueous  
Soln. is also prepared.  
However, it takes more  
time for complete  
dissolution than that  
the methanol soln. is  
prepared.

MnCl<sub>2</sub> Soln.

**Sol. F : 10 mmol/L MnCl<sub>2</sub>\* Solution**

**\*: Be careful not to confuse manganese with magnesium (Mg)**

- # MnCl<sub>2</sub>(H<sub>2</sub>O)<sub>4</sub> (MW: 198) ..... 0.10 g  
# Distilled water ..... 50 mL

Note: Do not use other anion salts such as Mn(NO<sub>3</sub>)<sub>2</sub> or Mn(CH<sub>3</sub>COO)<sub>2</sub>. White precipitates  
(Mn(OH)<sub>2</sub>) will be formed in basic aqueous solutions and gradually oxidize and turn  
brown (MnO(OH)), and the gel will be pigmented. Also, the functions of Mn<sup>2+</sup> will be  
deteriorated.

APS Solution

**Sol. G : 10% (w/v) Ammonium Persulfate Solution**  
**(Always prepare the Sol.G just before use.)**

- # (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (MW: 228) ..... 10 mg  
# distilled water ..... 0.10 mL

Wako Catalog No. 019-15922  
10 w/v% Ammonium  
Peroxisulfate Soln.  
(25 mL) Ready-to-use Sol. G



Running Buffer

**Sol. H : Running Buffer, pH 8.3 (10x soln.)**

- # Tris base (0.25 mol/L) ..... 15.1 g
- # SDS ..... 5.0 g
- # glycine (1.92 mol/L) ..... 72.0 g

Wako Catalog No. 184-01291  
Running Buffer Soln. (x10)  
(1 L)



⇒ Prepare the 0.50 L soln. by adding distilled water.  
Avoid to adjust the pH by adding acid or base.

**[Storage]** Keep at 4°C.

Just before use, add 450 mL  
of distilled water to 50 mL of Soln. H.

Wako Catalog No. 318-90323  
SDS-PAGE 10x Running Buffer  
(5 L)



Sample Buffer

**Sol. I : Sample Buffer (3x solution)**

- # Bromophenol Blue (BPB) ..... 1.5 mg
- # SDS ..... 0.60 g
- # glycerol ..... 3.0 mL
- # **Sol. C:** 0.50 mol/L Tris/HCl, pH 6.8 ..... 3.9 mL
- # 2-mercaptoethanol ..... 1.5 mL

Wako Catalog No. 191-13272  
Sample Buffer Soln.  
(2ME+)(x4) (25 mL)

Wako Catalog No. 196-11022  
Sample Buffer Soln.  
(2ME+)(x2) (25 mL)



⇒ Prepare the 10 mL soln. by adding distilled water.

**[Storage]** Keep at -20°C.

Usage of Soln. I: Please see the “④. Sample Preparation” (page #10).

Fixation Solution

**Sol. J : Acidic Solution for Fixation of Proteins (1 L)**

- # acetic acid ..... 0.10 L
- # methanol ..... 0.40 L
- # distilled water ..... 0.50 L

CBB Staining Soln.

**Sol. K : CBB Staining Solution (0.5 L)**

- # Coomassie Brilliant Blue (CBB) ..... 1.25 g
- # methanol ..... 0.20 L
- # acetic acid ..... 50 mL
- # distilled water ..... 0.25 L

Wako Catalog No. 174-00553  
Quick CBB Plus (250 mL)  
Wako Catalog No. 178-00551  
Quick CBB Plus (1 L)



*Applicable to silver  
staining and  
fluorescent staining.*

⇒ Dissolve CBB in methanol and then add acetic acid and water.

Washing and  
Destaining Soln.

**Sol. L : Washing and Destaining Solution (1 L)**

- # methanol ..... 0.25 L
- # acetic acid ..... 0.10 L
- # distilled water ..... 0.65 L

② Resolving Gel  
Preparation

*Note: Always  
prepare the gel  
just before use.*

**Resolving Gel Solution** (0.375 mol/L Tris, 0.1 mmol/L MnCl<sub>2</sub>, 0.1% SDS)

(In case of preparation of the 10 mL solution with 12 w/v% polyacrylamide gel and 50 μmol/L Phos-tag™ Acrylamide)

- # **Sol. A:** 30% (w/v) Acrylamide/Bis Mixed Solution ..... 4.00 mL
  - # **Sol. B:** 1.5 mol/L Tris/HCl Solution, pH 8.8 ..... 2.50 mL
  - # **Sol. E:** 5.0 mmol/L Phos-tag™ Solution ..... 0.10 mL
  - # **Sol. F:** 10 mmol/L MnCl<sub>2</sub> Solution ..... 0.10 mL \*1)
  - # **Sol. D:** 10% (w/v) SDS Solution ..... 0.10 mL
  - # TEMED (tetramethylethylenediamine) ..... 10 μL \*2)
  - # distilled Water ..... 3.14 mL
- ~Degas by stirring for 2 minutes.~
- # **Sol. G:** 10% (w/v) Ammonium Persulfate Solution ..... 50 μL \*2)

\*1) For the MnCl<sub>2</sub> solution,  
two times the Phos-tag™  
concentration (molar  
ratio) is added.

\*2) The conventional  
concentration can be  
used for TEMED and  
Sol.G. The above added  
amounts are examples.





**Attention**

Conditions should be examined for the amount of Solution E to be added. Please optimize Phos-tag™ acrylamide concentration and acrylamide concentration for each protein of interest. Please refer to page #17 for details.

**【Reference】** Examples of preparation of 10 mL of resolving gel solution

Phos-tag™ Acrylamide conc.	20 μM				50 μM				100 μM			
Acrylamide conc.	12%	10%	8%	6%	12%	10%	8%	6%	12%	10%	8%	6%
<b>Sol. A</b> (mL)	4	3.33	2.67	2	4	3.33	2.67	2	4	3.33	2.67	2
Sol. B (mL)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
<b>Sol. E</b> (mL)	0.04	0.04	0.04	0.04	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
<b>Sol. F</b> (mL)	0.04	0.04	0.04	0.04	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
Sol. D (mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TEMED (mL)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
<b>Distilled water</b> (mL)	3.26	3.93	4.59	5.26	3.14	3.81	4.47	5.14	2.94	2.65	4.31	4.98
Sol. G	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

**③ Stacking Gel Preparation**

\*2) The normally used concentration can be used for TEMED and Sol.G. The above added amounts are examples.

**Stacking Gel Solution** (0.125 mol/L Tris, 0.1% SDS)

(In case of preparation of 10 mL (2 mL) of 4.5% polyacrylamide gel.)

※The amount shown in parentheses are required for the 2 mL preparation.

# **Sol. A:** 30% (w/v) Acrylamide/Bis Mixed Solution ..... 1.50 mL (0.30 mL)

# **Sol. C:** 0.50 mol/L Tris/HCl Solution, pH 6.8 ..... 2.50 mL (0.50 mL)

# **Sol. D:** 10% (w/v) SDS Solution ..... 0.10 mL (20 μL)

# TEMED (tetramethylethylenediamine) ..... 10 μL (2 μL) \*2)

# distilled Water ..... 5.84 mL (1.17 mL)

~Degas by stirring for 2 minutes.~

# **Sol. G:** 10% (w/v) Ammonium Persulfate Solution ..... 20~50 μL (4~10 μL) \*2)

※Addition of SDS may not be necessary for resolving or stacking gels. Protein bands may become broad and tailing may occur in gels with SDS.

**② Preparation of Resolving Gel with low concentration containing agarose****<In case of Separation of 200~350 kDa Phosphorylated Proteins>**

By strengthening gels with 0.5% agarose, low concentration polyacrylamide gel at 3~5% can be prepared.

**Resolving Gel Solution** (0.375 mol/L Tris, 0.1 mmol/L MnCl<sub>2</sub>, 0.1% SDS)

(In case of preparation of 10 mL of 20 μmol/L Phos-tag™ Acrylamide containing 3.0% Polyacrylamide gel and 0.5% Agarose)

# **Sol. A:** 30% (w/v) Acrylamide/Bis Mixed Solution ..... 1.00 mL

# **Sol. B:** 1.5 mol/L Tris/HCl Solution, pH 8.8 ..... 2.50 mL

# **Sol. E:** 5.0 mmol/L Phos-tag™ Solution ..... 0.04 mL

# **Sol. F:** 10 mmol/L MnCl<sub>2</sub> Solution ..... 0.04 mL \*1)

# **Sol. D:** 10% (w/v) SDS Solution ..... 0.10 mL

# TEMED (tetramethylethylenediamine) ..... 10 μL \*2)

# distilled water ..... 2.93 mL

# 1.5% (w/v) agarose\*3) \*4) ..... 3.33 mL

# **Sol. G:** 10% (w/v) Ammonium Persulfate Solution ..... 50 μL \*2)

\*3) Add the agarose after distilled water has been added and thoroughly dissolved in a microwave oven and it is still hot.



\*4) If necessary, preheat the pipette tip and gel preparation table to 40 - 45°C.

Pour the agarose directly onto the gel preparation table before it hardens.

Agarose H (High Strength type)  
1 g (Wako Catalog No. 315-01203)  
10g (Wako Catalog No. 319-01201)  
25g (Wako Catalog No. 317-01202)



### ③ Preparation of Stacking Gel with low concentration containing agarose

#### Stacking Gel Solution (0.125 mol/L Tris, 0.1% SDS)

(In case of preparation of 10 mL (or 2 mL) of 3.0 (w/v)% polyacrylamide containing 0.5%(w/v) agarose.)

# Sol. A:	30% (w/v) Acrylamide/Bis Mixed Solution	1.00 mL (0.20 mL)
# Sol. C:	0.50 mol/L Tris/HCl Solution, pH 6.8	2.50 mL (0.50 mL)
# Sol. D:	10% (w/v) SDS Solution	0.10 mL (20 μL)
# TEMED	(tetramethylethylenediamine)	10 μL (2 μL) *2)
# distilled Water		3.01 mL (602 μL)
# 1.5% (w/v) agarose	*3) *4)	3.33 mL (666 μL)
# Sol. G:	10% (w/v) Ammonium Persulfate Solution	50 μL (10 μL) *2)



Pour the agarose directly onto the gel preparation table before it hardens.

### ④ Sample Preparation

- 1) Mix sample with 3 μL of **Solution I** (Sample Buffer) and add an appropriate amount of distilled water to make 9 μL solution in a microcentrifuge tube.
- 2) Heat at 95°C for 5 minutes, then, allow the solution to cool to room temperature.
- 3) Load the sample solution (eg: 1.5 μL/well) using a micropipette.  
※ In case of β-casein, load 5~10 μg /well to obtain clear bands.  
※ It is highly recommended that impurities be removed using TCA precipitation or dialysis, etc.

### ⑤ Electrophoresis

- 1) Assemble the electrophoresis equipments and fill the electrode chambers with **Solution H** (Running Buffer).
- 2) Gently remove the comb from the stacking gel and load the sample into each well using a micropipette.
- 3) Attach the leads to the power supply. Run the gel under a constant current condition (25~30 mA/gel) until the BPB reaches the bottom of the resolving gel. (※In case of two gels, run the gels at 50~60 mA.)

※When performing Western blotting or mass spectrometry, please refer to the page #13 below after electrophoresis.

### ⑥ CBB Staining •Destaining

- 1) Just after electrophoresis, the gel is soaked in 50 mL of the **Sol. J** (Acidic Solution for Fixation of Proteins) for ca. 10 min. with gentle agitation.
- 2) Stain the gel by soaking in 50 mL of the **Sol. K** (CBB Staining Solution) for ca. 2 hours with gentle agitation.
- 3) Wash the gel in 50 mL of the **Sol. L** (Washing and Destaining Solution) 3 times to remove excess stain until the background is sufficiently clear.
- 4) Take a picture of the gel.

## [ II ] Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE ※Gels are usable up to three months after casting.

**NEW!**

Though the Mn<sup>2+</sup>-Phos-tag™ SDS-PAGE adopts the typical Laemmli SDS-PAGE method and is a simple procedure, there have been cases where separation of phosphorylated and non-phosphorylated forms was not possible, depending on the protein. In contrast, Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE using the neutral Bis-Tris gel SDS-PAGE system dramatically improves resolution, since Phos-tag™ shows the highest phosphoric acid group-capturing ability at neutral pH.

### ① Preparation of reagents

Acrylamide Solution

SDS Solution

Phos-tag™

Acrylamide Solution

APS Solution

ZnCl<sub>2</sub> Solution

10 mmol/L Zn(NO<sub>3</sub>)<sub>2</sub>  
is also usable.

Bis-Tris/HCl Solution

Sodium Bisulfite  
Solution

Running Buffer

\*1) Add ZnCl<sub>2</sub> with double volume (molar ratio) of Phos-tag™ Acrylamide.

\*2) The normally used concentration can be used for TEMED and Sol.G. The above added amounts are examples.



Ready-to-use reagents are available. Refer to page #29~.



**Sol. A:** 30% (w/v) Acrylamide/Bis Mixed Solution (30% T, 3.3% C)



**Sol. D:** 10% (w/v) SDS Solution



**Sol. E:** 5.0 mmol/L Phos-tag™ Solution containing 3% (v/v) methanol



**Sol. G:** 10% (w/v) Ammonium Persulfate Solution

Please refer to [ I ]  
Mn<sup>2+</sup>-Phos-tag™  
SDS-PAGE.

**Sol. M:** 10 mmol/L ZnCl<sub>2</sub> Solution **Note:**Please prepare it just before use.

ZnCl<sub>2</sub> (MW: 136, 98+%) ..... 0.70 g

distilled water ..... 500 mL

※As ZnCl<sub>2</sub> is deliquescent, please use it fresh. If impurities such as ZnO can be seen, please remove it using filtration.

**Sol. N:** 1.4 mol/L Bis-Tris/HCl Solution, pH 6.8 (4x solution for resolving gel)

Bis-Tris base (MW: 209, pKa = 6.5 at 20°C) ..... 29.9 g

6.0 mol/L HCl (0.42 equivalent of Bis-Tris) ..... 10 mL

Prepare the 100mL solution by adding distilled water.[storage:keep at 4°C in the dark.]

**Sol. O:** 0.5 mol/L Sodium Bisulfite Solution

NaHSO<sub>3</sub> (FW: 106) ..... 5.3 g

Prepare the 100mL solution by adding distilled water.[storage:keep at 4°C in the dark.]  
(Keep away from air.)

**Sol. P:** Running Buffer, pH 7.8 (5x solution)

Tris base (FW: 121, pKa = 8.2 at 20°C, 0.50 mol/L) ..... 30.3 g

MOPS (FW: 209, pKa = 7.2 at 20°C, 0.50 mol/L) ..... 52.3 g

**Sol. D:** 10% (w/v) SDS Solution (0.5% (w/v)) ..... 25 mL

Prepare the 500mL solution by adding distilled water. Do not adjust the pH.  
[storage:keep at 4°C in the dark.]

**Running Buffer** **Note:**Please prepare just before use.

**Sol. P:** Running Buffer, pH 7.8 (5x solution) ..... 100 mL

**Sol. O:** 0.5 mol/L Sodium Bisulfite Solution ..... 5 mL

Prepare the 500mL solution by adding distilled water.

### Resolving Gel Solution

(In case of preparation of 10mL gel with 12% acrylamide gel, 50 μmol/L Phos-tag™ Acrylamide and 100 μmol/L ZnCl<sub>2</sub>)

**Sol. A:** 30% (w/v) Acrylamide Solution ..... 4.00 mL

**Sol. N:** 1.4 mol/L Bis-Tris/HCl Solution, pH 6.8 ..... 2.50 mL

**Sol. E:** 5.0 mmol/L Phos-tag™ Solution ..... 0.10 mL

**Sol. M:** 10 mmol/L ZnCl<sub>2</sub> Solution ..... 0.10 mL \*1)

TEMED (tetramethylethylenediamine) ..... 10 μL \*2)

distilled water ..... 3.24 mL

Degas by stirring for 2minutes.

**Sol. G:** 10% (w/v) Ammonium Persulfate Solution ..... 50 μL \*2)

**Attention**

Conditions should be examined for the amount of Solution E to be added. Please optimize Phos-tag™ acrylamide concentration and acrylamide concentration for each protein of interest. Please refer to page #17 for details.



### Stacking Gel Solution

(In case of preparation of 10mL(2mL) of 4.5% acrylamide gel)

※The amount shown in parentheses are required for the 2mL preparation.

**Sol. A** : 30% (w/v) Acrylamide/Bis Mixed Solution ..... 1.50 mL (0.30 mL)

**Sol. N** : 1.4 mol/L Bis-Tris/HCl Solution, pH 6.8 ..... 2.50 mL (0.50 mL)

TEMED (tetramethylethylenediamine) ..... 10  $\mu$ L (2  $\mu$ L) \*2)

distilled water ..... 5.94 mL (1.19 mL)

Degas by stirring for 2 minutes.

**Sol. G** : 10% (w/v) Ammonium Persulfate Solution ..... 50  $\mu$ L (10  $\mu$ L) \*2)

※SDS addition is not necessary in resolving or stacking gels.

Please refer to [1] for sample preparation, electrophoresis, Coomassie Brilliant Blue staining and destaining. Depending on the protein, band "smiling" may be observed. One of the reasons is that the protein's metal ligand (such as a thiol group) causes Zn<sup>2+</sup> dissociation from the Phos-tag™ molecule. Adding ZnCl<sub>2</sub> to a final concentration of 1mM in the sample buffer will reduce smiling and improve resolution.

## <Separation of large phosphorylated proteins bigger than 200kDa>

In conventional Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE, Bis-Tris is used in the resolving and stacking gels. Bis-Tris acts as a radical quencher since its structure is similar to TEMED. As a result, the bands smear due to a lost in sieving effect in low concentration polyacrylamide gels (less than 5%). If performing Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE using 3% or 4% low concentration polyacrylamide, please prepare gels with Tris-AcOH in place of Bis-Tris. Please refer to [1]-③ as well. Recommended for large proteins above 200kDa.

Composition	Tris-AcOH gel	Bis-Tris gel
Running buffer	• 50mM Tris • 50mM Tricine • 0.1%(w/v) SDS • 5.0mM Sodium Bisulfite	• 100mM Tris • 100mM MOPS • 0.1%(w/v) SDS • 5.0mM Sodium Bisulfite
Resolving / Stacking buffer	• 200mM Tris-AcOH (pH 7.0)	• 357mM Bis-Tris-HCl (pH 6.8)
Sample buffer	• Conventional Laemmli System	• Conventional Laemmli System
Gel concentration	• 3~4 % Polyacrylamide + 0.5 % Agarose	• ≥ 5 % Polyacrylamide

## ■ Post-Phos-tag™ SDS-PAGE analysis

### ◆ Western Blotting

**NOTE**

When transferring phosphorylated forms from Phos-tag™ SDS-PAGE gel to the membrane, transferring rate tends to decrease considerably. In order to improve the transfer rate, it is necessary to remove  $Mn^{2+}$  and  $Zn^{2+}$  using EDTA. Please carry out the following depending on the type of transfer apparatus used.

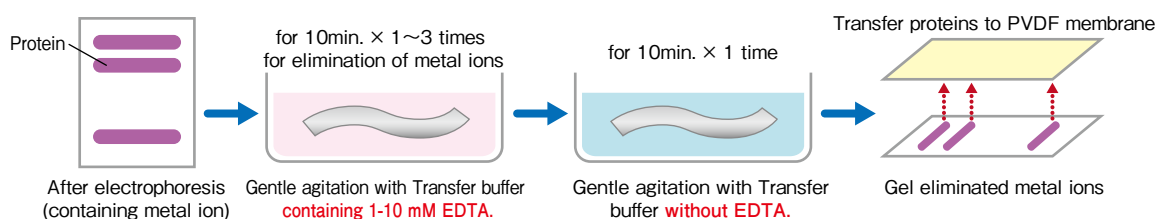
#### ① Using the semi-dry method

(1) After electrophoresis, please immerse the gel in transfer buffer containing 10 mmol/L EDTA and shake gently. (1 to 3 times for 10 min each)

※Here is an example of EDTA concentration, processing time, frequency of EDTA-containing buffer changing. Please optimize conditions as necessary.

※Please change the EDTA-containing buffer depending on gel thickness. (Example: 1.5 mm thickness: 2 times for 20 min)

(2) Next, please immerse the gel in transfer buffer without EDTA.



**Attention**

※Please optimize blotting conditions such as time and temperature for the target protein.

When using highly concentrated  $Zn^{2+}$ -Phos-tag™ gels (example: 100  $\mu M$  Phos-tag™), sufficient transfer rate may not be attained even after EDTA treatment. If such is the case, please use the tank method.

#### ② Using the tank method (wet)

Please use transfer buffer containing 0.1% SDS. When using the tank method, the EDTA treatment step can be omitted as long as SDS-containing transfer buffer is used. Pay careful attention as proteins may fall off the membrane. Please perform the procedures using SDS at the optimal concentration of 0.05% to 0.2%

Wako Cat. No. 019-25111  
**AquaBlot™ 10 × Tris-Glycine-SDS Transfer Buffer (1L)**

### ◆ Quantification analysis

Please perform silver staining and Coomassie Brilliant Blue staining after electrophoresis, and then follow the usual in-gel digestion protocol to prepare samples. EDTA treatment procedures are not necessary.

Wako Cat. No.  
299-58901  
**Silver Stain MS kit**  
(20 tests)



# 4. Trouble Shooting

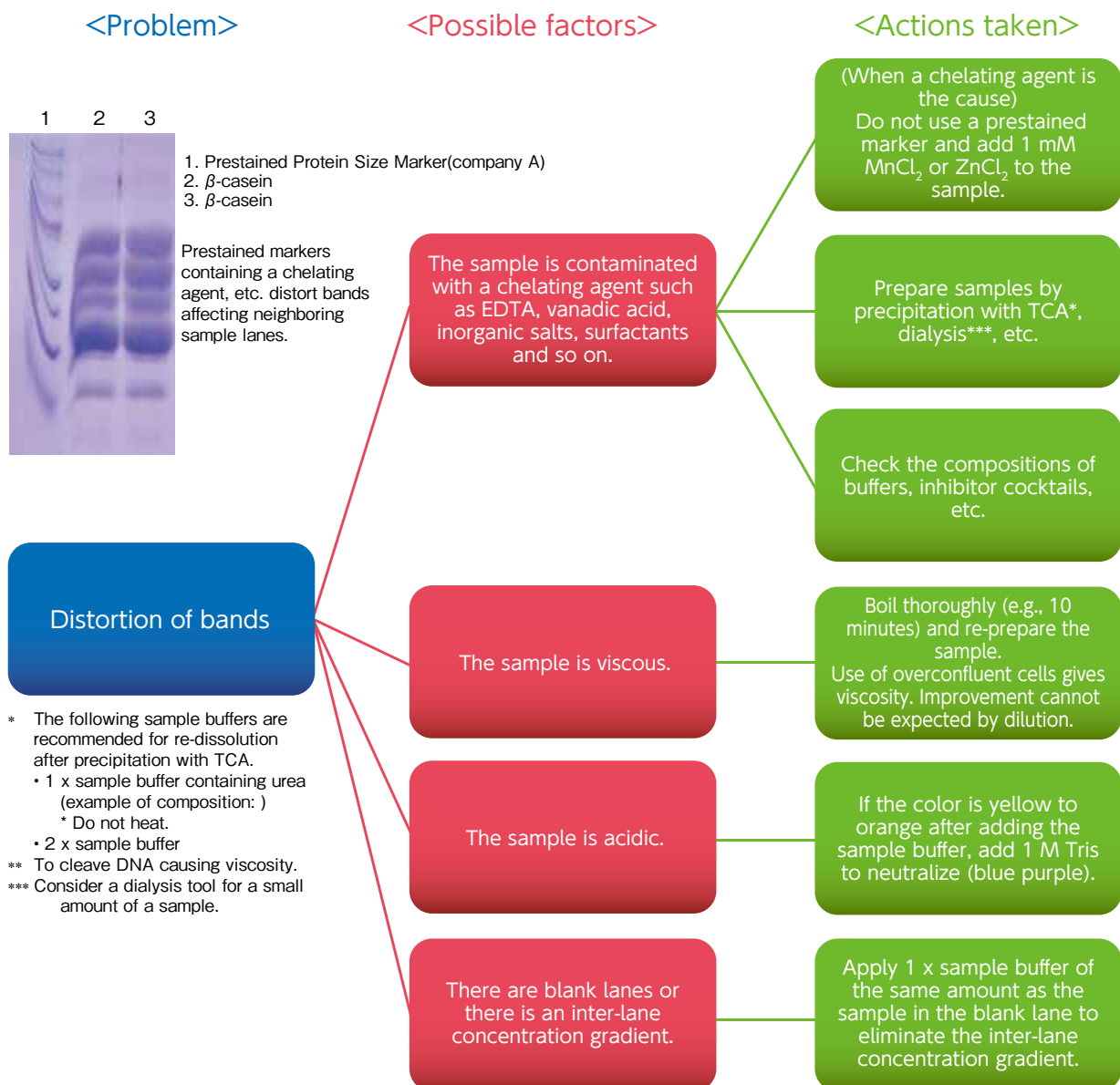
## Introduction – Key to success is "preparation of samples" –

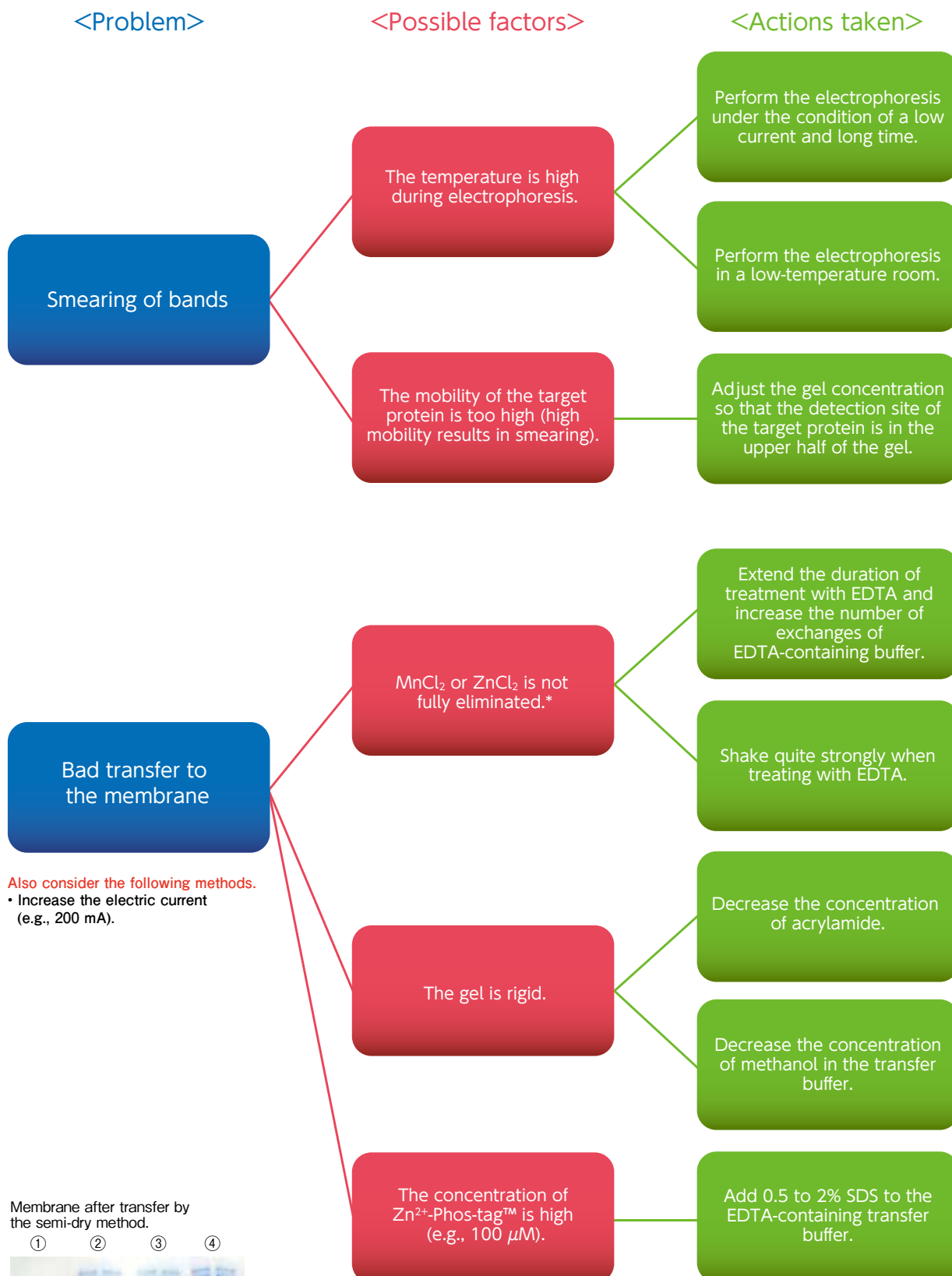
The sample condition makes a significant difference. Prepare samples keeping the following points in mind:

- Contamination of influential substances: In particular, never use EDTA. Make sure that commercially available inhibitor cocktails and buffers do not contain EDTA. Also eliminate EDTA from constituents of media.
- Sample condition: Do not use highly viscous samples such as overconfluent cell lysates (dilution does not improve much).
- Standardization of buffer composition for samples: Standardize the composition of the buffer for each sample to be applied to the gel. Pay particular attention when samples and markers treated with phosphatase are used. Apart from chelating agents and surfactants, concentrations of  $MnCl_2$  and  $ZnCl_2$  have some influences.

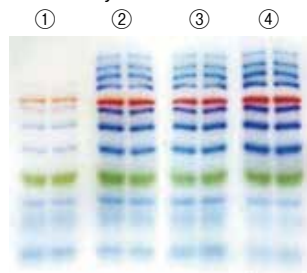
If the preparation has a trouble, it is advised to re-prepare the sample paying attention to the above.

## Popular trouble shootings (causes and actions taken)





Membrane after transfer by the semi-dry method.

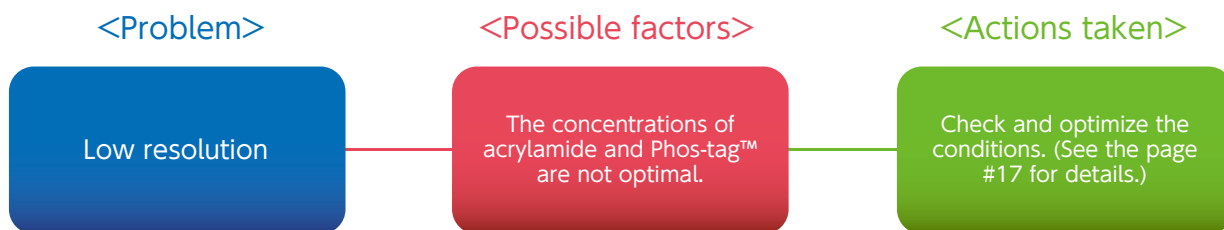


- ① No EDTA treating
- ② 1mM EDTA for 10 minutes × 2 times
- ③ 10mM EDTA for 10 minutes × 1 times
- ④ 1mM EDTA for 10 minutes × 2 times

**[Sample]** WIDE-VIEW™ Prestained protein Size Marker III(Wako Cat. No.230-02461, etc.)

**[Gel]** SuperSep™ Phos-tag™ (50μmol/L),12.5%, 13 wells (wako Cat. No. 196-16701)

\* The transfer rate decreases considerably if EDTA treatment is not performed (by comparison of ① with ②, ③ and ④). First of all, It is advised to wash twice with 1-10 mM EDTA each for 10 minutes. In particular, duration of the treatment and number of exchanges of EDTA-containing buffer are important.



Also consider the following methods.

- Increase the molar ratio of metal ion ( $\text{MnCl}_2$  or  $\text{ZnCl}_2$ ) to Phos-tag™ acrylamide in the gel (e.g. 1:4).
- Use Tris-Tricine buffer as the running buffer.
- (In the case of  $\text{Mn}^{2+}$ -Phos-tag™ SDS-PAGE) Consider  $\text{Zn}^{2+}$ -Phos-tag™ SDS-PAGE.
- Prepare the reagents freshly.

Wako Cat. No. 200-17071  
Tricine Running Buffer  
Solution (×10) (1 L)



## Other trouble shootings

- ◆ Phosphorylated form is not obtained from immunoprecipitation samples.  
When a monoclonal antibody is used, a phosphorylated form may not be obtained due to overlapping of the epitope and the phosphorylated region. For purification of the target protein by the immunoprecipitation, it is advised to use a polyclonal antibody.
- ◆ The sample condition is unusual.  
Washing with PBS in the preparation of cell lysate may affect the phosphorylation condition. Add TCA immediately after elimination of the medium.
- ◆ It is difficult to judge whether the gel or the sample is problematic.  
A mixture of phosphorylated  $\alpha$ -casein and dephosphorylated  $\alpha$ -casein is available as a positive control. Perform the ordinary SDS-PAGE simultaneously using this product as a sample.
- ◆ Phos-tag™ acrylamide does not dissolve.  
Warming at about 40°C or treatment with an ultrasonic bath after addition of methanol and water makes dissolution easier.
- ◆ Separation and migration speed vary among lanes.  
The concentration of the substance interfering with Phos-tag™ (such as EDTA,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ) may differ considerably depending on the sample. Prepare samples taking care not to vary the concentration among them.

## Protein Diffusion

Long-term migration with a constant current will cause decomposition and diffusion of proteins due to excessive heat.

- ① If you want to use a constant current for migration, try techniques such as using a low-temperature room, thoroughly cooling the migration buffer just before use, and wrapping a cooling agent around the migration tank (but do not use ice because it may cause electric shock).
- ② When a constant voltage can also be used, migrate with a constant voltage (eg: 200 V). The migration speed will slow down but the generation of heat will be suppressed.

## Easy breaking of the gel

The gel is softened due to the low concentration of acrylamide.

- ① 5% or higher : Increasing the *N,N'*-methylene-bisacrylamide to acrylamide ratio (eg: 24 : 1) will strengthen the gel.
- ② Add 3~5% of agarose to strengthen gels. Refer to "Preparing a Low-Concentration Gel Containing Agarose" in "3. Protocol" and "Separation" in "7. FAQ."

## The background becomes high after staining.

Carry out staining after eliminating metal ions in the gel by EDTA treatment similarly to the Western blotting.





## 5. Optimization of Phos-tag™ SDS-PAGE Condition

To obtain a high quality result using Phos-tag™ SDS-PAGE, optimization of the concentration of acrylamide and Phos-tag™ Acrylamide is essential. Optimize the concentration of acrylamide (①) first, followed by that of Phos-tag™ Acrylamide (②).

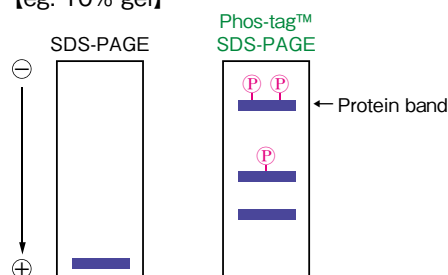
### ① Optimization of the concentration of acrylamide

First, identify the optimum concentration of acrylamide\* that allows migration of the target protein to the lowest end of the gel when conventional SDS-PAGE is used.

In Phos-tag™ SDS-PAGE, the migration speed is slower than in conventional SDS-PAGE (including non-phosphorylated proteins) and, therefore, the concentration of acrylamide should be examined (see the below figure). The migration speed decreases as the concentration of Phos-tag™ increases.

\*: Run a gel electrophoresis until the BPB dye, which is contained in the sample buffer, reaches the bottom of the resolving gel. The position of BPB dye can be defined as an Rf value of 1.0. Under the above mentioned running condition, adjust the optimum concentration of acrylamide. When your target protein is observed as a migration band at an Rf value of 0.8 to 0.9 in conventional SDS-PAGE, the acrylamide concentration would be optimal for Phos-tag™ SDS-PAGE.

[eg: 10% gel]



#### Indication of optimum concentration

more than 60 kDa: 6%    less than 60 kDa : 8%  
<In case of high molecular weight proteins >

The gel strength can be increased by adding agarose to gels that contain less than 4% of acrylamide. There are data of separation of 350 kDa. (Refer to "Separation of Phos-tag™ Acrylamide" of 7. FAQ) Furthermore, the gel strength can also be enhanced by increasing the *N,N'*-methylenebisacrylamide content (eg: 5% acrylamide [24:1]).

### ② Optimization of the concentration of Phos-tag™ Acrylamide

First of all, start with a lower concentration of Phos-tag™ acrylamide at 20  $\mu\text{M}$ , raise the concentration up to 100  $\mu\text{M}$ , and select the concentration at which the difference in mobility between the phosphorylated form and nonphosphorylated form is large. Supplier of information:

Then, optimize the concentration of Phos-tag™ Acrylamide. Please evaluate the optimum concentration in the order of lowest to highest.

eg: 20 $\mu\text{M}$ →50 $\mu\text{M}$ →100 $\mu\text{M}$

[Cell Lysate]

In case there is a large variety of proteins in your sample, eg: cell lysates, the concentration of Phos-tag™ should be 5 to 25  $\mu\text{M}$ . However, a higher concentration, eg: 100  $\mu\text{M}$ , is recommended in case of a lower concentration of the target protein, eg: non-overexpression systems.

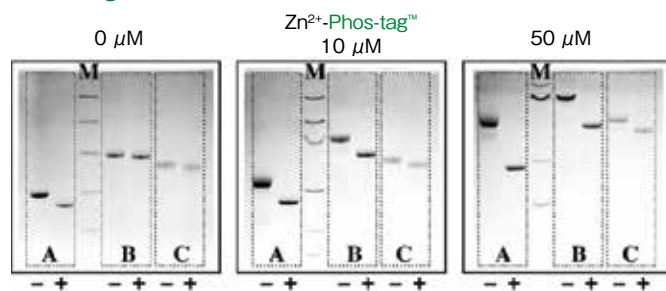
※ The optimum condition depends on the protein. Please find the appropriate condition setting for each target protein.

(The information was provided by Yasunori Sugiyama Kagawa University)

#### Relation between Phos-tag™ concentration and resolution/mobility

In general, a higher concentration leads to higher separation capacity. (Compare the samples of 50  $\mu\text{M}$  and 100  $\mu\text{M}$  of  $\text{Mn}^{2+}$ -Phos-tag™ in the left figure.) However, the higher concentration causes low velocity. It sometimes happens that the higher separation capacity is due to the lower Phos-tag™ concentration (Compare the samples of 50  $\mu\text{M}$  and 150  $\mu\text{M}$  of ovalbumin of the right figure.)

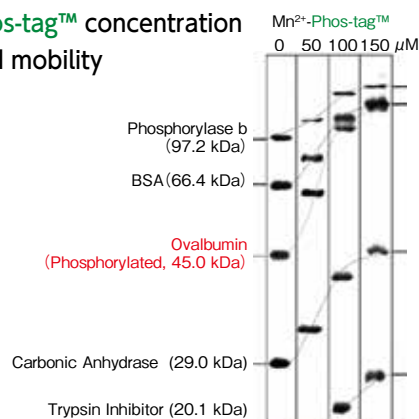
#### [Phos-tag™ concentration and resolution]



A :  $\beta$ -casein, B: Ovalbumin, C: Pepsin, M: Marker  
+ : Phosphorylated proteins (A: 5 $\text{P}$ , B: 2 $\text{P}$ , C: 1 $\text{P}$ )  
- : De-phosphorylated proteins (Alkaline phosphatase treated)

Phos-tag™ conc. (High) → Separation Capacity (High)

#### Phos-tag™ concentration and mobility



Phos-tag™ conc. (High) → Migration degree (Low)

# 6. Application Data and References

## Application Data 1

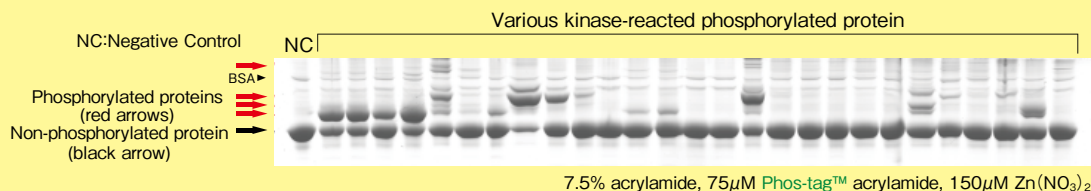
Impressions of users of Phos-tag™ Acrylamide and SuperSep Phos-tag™ precast gels are introduced herein. 'Ogawa provided comments and data of the sample, which had been assayed for kinase activity, separated by Phos-tag™ SDS-PAGE. Kimura provided the application data to a secondary electrophoresis, and Hosokawa provided the application data to the western blotting from Professor Tadayuki Ogawa of the University of Tokyo. In addition, we received data applied to two-dimensional electrophoretic migration from Professor Yayoi Kimura of Yokohama City University, as well as Western blotting applied data from Professor Sugiyama of Kochi University and Professor Hosokawa of RIKEN.

**"I recommend Phos-tag™."**

Tadayuki Ogawa, Graduate School of Medicine, the University of Tokyo

Phos-tag™ is a very convenient reagent that can be applied in a variety of samples and research purposes. It allows quantitative analysis not only of in vitro assay samples but also in vivo samples in a phosphorylated state. Phos-tag™ SDS-PAGE utilizes normal electrophoretic migration and does not require the purchase of special equipment, so you could say it has good cost performance. Phosphorylation research that used to require anti-phosphorylated antibodies, RI, and many other reagents will now be advanced with Phos-tag™.

### Comparison between a phosphorylated protein and a non-phosphorylated protein by Phos-tag™ SDS-PAGE

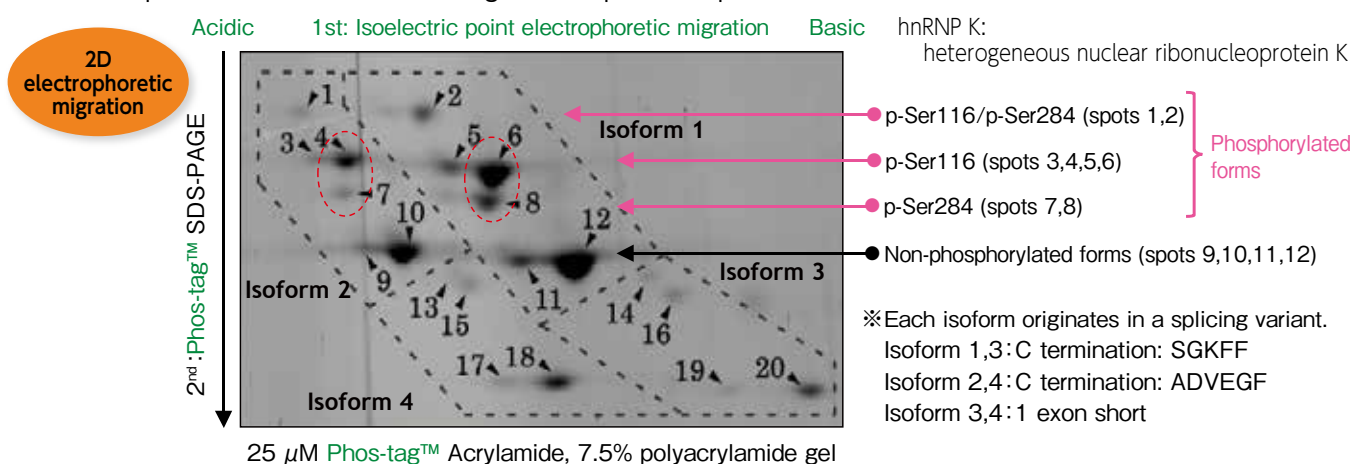


In order to explore a kinase that phosphorylates about 40 kDa protein, the samples assayed for kinase activity were separated by Phos-tag™ SDS-PAGE. A lot of information was obtained from a small amount of samples as, compared to nonphosphorylated samples (NC), the proportion of phosphorylation/nonphosphorylation, degree of phosphorylation and distribution of population differed by the type of the kinase that reacted with other samples. With this information as the foothold, more detailed analyses using mass spectrometry, etc. were performed to identify the phosphorylation site specific to each kinase. (Reference : Ogawa T., Hirokawa N., *Cell Rep.*, 2015 Sep. 22 ; 12(11) : 1774-88)

## Application Data 2

### Application in two-dimensional electrophoretic migration: Analysis of phosphorylated isoforms of hnRNP K

hnRNP K was separated by immune precipitation from a 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. Isoforms and modification sites of the various spots were then identified using a mass spectroscope.



**Different spots of phosphorylated states were detected at the same isoelectric point for each isoform! (eg: 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.

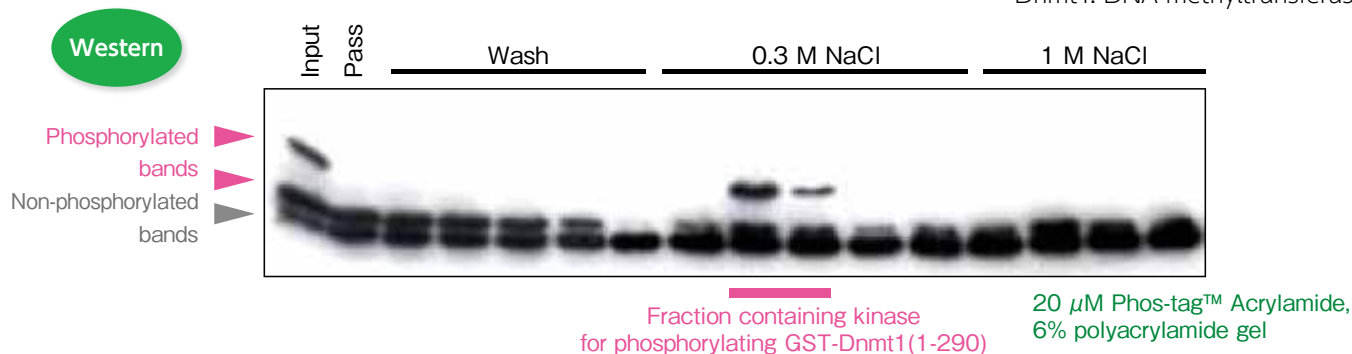
Data provided by: Professors Y. Kimura and H. Hirano, Biological Supramolecular Systems, Graduate School of Bionano-systems, Yokohama City University; and Professor O. Ohara, RCAI, Physics and Chemistry Research Institute.



## Application Data 3

### Determining fraction containing kinase for phosphorylating Dnmt1

Dnmt1: DNA methyltransferase



- 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 Dnmt1 (72-86))

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

Data published in :

The DNA-binding activity of mouse DNA methyltransferase 1 is regulated by phosphorylation with casein kinase 1 delta/epsilon. 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.

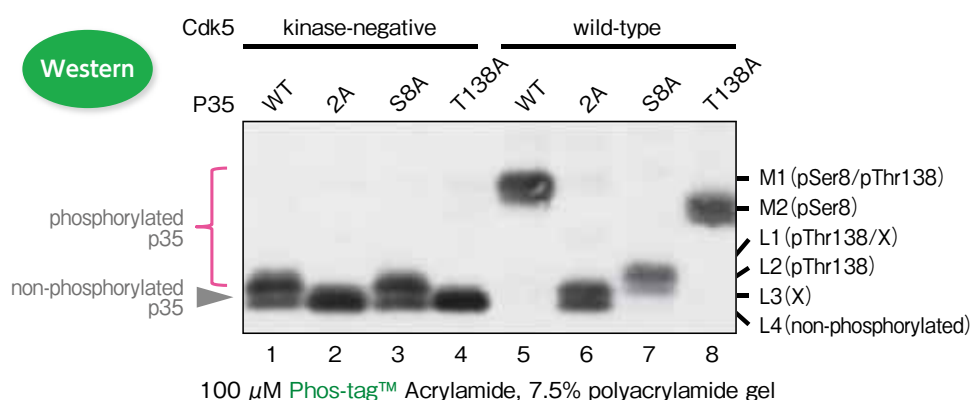
Data provided by: Professor Y. Sugiyama, and Professor I. Kameshita, Department of Life Science, Faculty of Agriculture, Kagawa University.

## Application Data 4

### Search for phosphorylation site of Cdk5-activated sub-unit p35 using Ala substitution variant

Cdk5: cyclin-dependent kinase 5

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)



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

Relationship between phosphorylation site and band shift was clarified!

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: Professor T. Hosokawa, Memory Mechanisms Research Team, Circuit Function Mechanism Core, Brain Science Research Center, RIKEN; and Professor S. Hisanaga, Nerve Molecular Functions Research Room, Bio-science Studies, Institute of Science & Engineering, Tokyo Metropolitan University.



## References

### Regarding Phos-tag™ reagents:

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2. Phosphate-binding tag: A new tool to visualize phosphorylated proteins, *Molecular & Cellular Proteomics*, **5**, 749-757 (2006), E. Kinoshita, E. Kinoshita-Kikuta, K. Takiyama, and T. Koike
3. Separation and detection of large phosphoproteins using Phos-tag™ SDS-PAGE, *Nature Protocols*, **4**, 1513-1521 (2009), E. Kinoshita, E. Kinoshita-Kikuta, and T. Koike

### Application using Mn<sup>2+</sup>- Phos-tag™ SDS-PAGE

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3. Dbf4-Dependent Cdc7 Kinase Links DNA Replication to the Segregation of Homologous Chromosomes in Meiosis I, *Cell*, **135**, 662-678 (2008), J. Matos, J. J. Lipp, A. Bogdanova, S. Guillot, E. Okaz, M. Junqueira, A. Shevchenko, and W. Zachariae
4. Kinome Profiling in Pediatric Brain Tumors as a New Approach for Target Discovery, *Cancer Res.*, **69**, 5987-5995 (2009), A. H. Sikkema, S. H. Diks, W. F.A. den Dunnen, A. ter Elst, F. J.G. Scherpen, E. W. Hoving, R. Ruijtenbeek, P. J. Boender, R. de Wijn, W. A. Kamps, M. P. Peppelenbosch, and E. S.J.M. de Bont
5. Regulation of mitochondrial transport and inter-microtubule spacing by tau phosphorylation at the sites hyperphosphorylated in Alzheimer's disease, *J. Neurosci.*, **32**, 2430-2441 (2012), K. Shahpasand, I. Uemura, T. Saito, T. Asano, K. Hata, K. Shibata, Y. Toyoshima, M. Hasegawa, S. Hisanaga
6. The Hsp90 Kinase Co-chaperone Cdc37 Regulates Tau Stability and Phosphorylation Dynamics, *J. Biol. Chem.*, **286**, 16976-16983 (2011), Umesh K. Jinwal, Justin H. Trotter, Jose F. Abisambra, John Koren, III, Lisa Y. Lawson, Grant D. Vestal, John C. O' Leary, III, Amelia G. Johnson, Ying Jin, Jeffrey R. Jones, Qingyou Li, Edwin J. Weeber, and Chad A. Dickey

Tau proteins are used as the samples in Application 5 and 6.

7. PINK1-Phosphorylated Mitofusin 2 Is a Parkin Receptor for Culling Damaged Mitochondria, *Science*, Apr 2013; **340**: 471 - 475., Yun Chen and Gerald W. Dorn, II
8. Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice, *Science*, Dec 2015; **350**: aad2459., Guohua Gong, Moshi Song, Gyorgy Csordas, Daniel P. Kelly, Scot J. Matkovich, and Gerald W. Dorn, II

### Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE

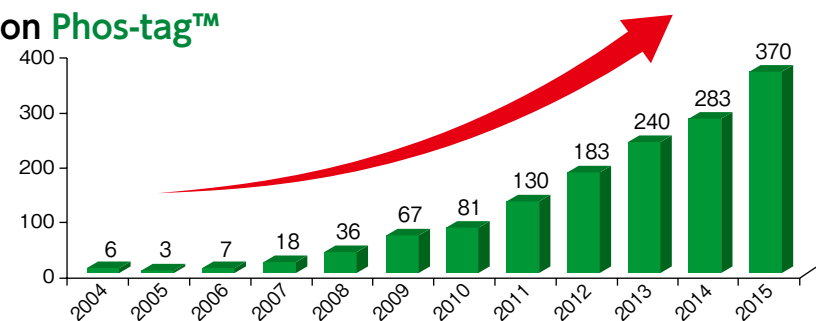
1. Phosphorylation of Phytochrome B Inhibits Light-Induced Signaling via Accelerated Dark Reversion in Arabidopsis, *PLANT CELL*, Feb 2013; **25**: 535 - 544., Mátyás Medzihradszky, János Bindics, Éva Ádám, András Viczián, Éva Klement, Séverine Lorrain, Péter Gyula, Zsuzsanna Mérai, Christian
2. MAPK feedback encodes a switch and timer for tunable stress adaptation in yeast, *Sci. Signal.*, Jan 2015; **8**: ra5., Justin G. English, James P. Shellhammer, Michael Malahe, Patrick C. McCarter, Timothy C. Elston, and Henrik G. Dohlman
3. Mechanism of Activity-Dependent Cargo Loading via the Phosphorylation of KIF3A by PKA and CaMKIIa., *Neuron*. 2015 Sep 2; **87**(5):1022-35., Ichinose S, Ogawa T, and Hirokawa N.
4. Microtubule Destabilizer KIF2A Undergoes Distinct Site-Specific Phosphorylation Cascades that Differentially Affect Neuronal Morphogenesis, *Cell Reports*, 2015 Sep 22; **12**(11):1774-88

### SuperSep™ Phos-tag™

1. A laborsaving, timesaving, and more reliable strategy for separation of low-molecular-mass phosphoproteins in Phos-tag affinity electrophoresis. *Int. J. Chem.* **4**, 1-8 (2012), Kinoshita-Kikuta, E., Kinoshita, E., and Koike, T.
2. In vivo collective cell migration requires an LPAR2-dependent increase in tissue fluidity, *J. Cell Biol.*, Jul 2014; **206**: 113 - 127., Sei Kuriyama, Eric Theveneau, Alexandre Benedetto, Maddy Parsons, Masamitsu Tanaka, Guillaume Charras, Alexandre Kabla, and Roberto Mayor
3. DNA replication and spindle checkpoints cooperate during S phase to delay mitosis and preserve genome integrity, *J. Cell Biol.*, Jan 2014; **204**: 165 - 175., Maria M. Magiera, Elisabeth Gueydon, and Etienne Schwob

## Increasing number of articles on Phos-tag™

Phos-tag    
by Google Scholar



# 7. FAQ

## Phos-tag™ Acrylamide

### Quantification

Q. Can phosphorylated proteins be quantified?

A. They can be assayed on the basis of the band intensity by using a quantitative staining such as CBB staining. A product such as "Quick-CBB PLUS" is recommended.

⇒ Quick-CBB PLUS (1 L: Wako Cat. #178-00551; 250 mL: 174-00553)

### Separation

Q. Is there a limitation to the protein size for the application of Phos-tag™?

A. A phosphorylated protein of 350 kDa has actually been separated with 20  $\mu$ M Phos-tag™, 3% acrylamide and 0.5% agarose\*. (See "[1]-②", ③ in "3. protocol" and "Tris-AcOHgel" on page 12 for details.)

Reference: *Proteomics*, 9, 4098-4101 (2009), E. Kinoshita, E. Kinoshita-Kikuta, H. Uchijima, and K. Koike

\* Agarose was added to strengthen the gel.

### Staining

Q. Is it possible to use gel-staining techniques other than CBB?

A. Yes, the gel can also be stained by negative staining, silver staining, and fluorescent staining.

### Use of Phos-tag™ Acrylamide

Q. How many gels can be made with each product?

A. It depends on the concentration of Phos-tag™ used.

For example, about 100 plates at 20  $\mu$ M, about 40 plates at 50  $\mu$ M, and about 20 plates at 100  $\mu$ M can be prepared from a 10 mg-package, when gels of 1 mm-thickness, 9 cm-width, and 7.7 cm-length are made.

Phos-tag™	20 $\mu$ M	50 $\mu$ M	100 $\mu$ M
0.3 mL Aqueous Soln. (0.9 mg)	abt. 9	abt. 4	abt. 2
2 mg package	abt. 20	abt. 8	abt. 4
10 mg package	abt. 100	abt. 40	abt. 20
SuperSep Phos-tag™	—	5 gels	—

### Gel Strength

Q. The gel is too soft. What can I do for this?

A. Please refer to "4. Trouble Shooting (page #14).

### Stability of the prepared gel containing Phos-tag™

Q. How long can the prepared gel containing Phos-tag™ Acrylamide be stored?

A. Mn<sup>2+</sup>-Phos-tag™ SDS-PAGE gel cannot be stored. Use the gel on the day after preparation.

Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE gel can be stored in a refrigerator for 3 months.

The gel deteriorates within a few days. Therefore, it should be prepared just before use.

### Stability of the Phos-tag™ solution

Q. How long can the solutions in methanol and water be stored?

A. No remarkable decline in performance has been reported for 1 year by refrigeration under protection from light.

## Phos-tag™ Acrylamide (continued)

### Preparation of the reagent

Q. Does the concentration of Phos-tag™ influence the amount of ions to be required?

A. The molar ratio of Phos-tag™ acrylamide to  $Mn^{2+}$  should be 1:2; two  $Mn^{2+}$  ions bind to one Phos-tag™ molecule (Fig. 1).

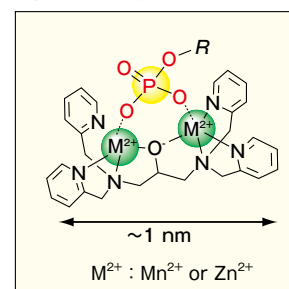
Q. I have experienced clouding of Phos-tag™ when I prepared a solution as described in the protocol. Is this normal?

A. Yes, it is. Clouding is attributed to methanol. The solution becomes clear after standing for a while.

Q. Does Phos-tag™ dissolve in water alone?

A. It is soluble in water, though it takes more time compared to dissolution in water containing methanol. If it does not dissolve completely, centrifuge the solution and use the supernatant.

Fig. 1 Phos-tag™ Basic Structure

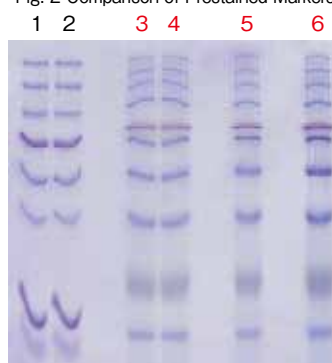


### Molecular marker

Q. What prestained markers can we use?

A. Using a prestained marker with the Phos-tag™ gel usually causes distortion of bands (Fig. 2). WIDE-VIEW™ Prestained Protein Size Marker III (Wako Cat No. 230-02461) is less likely to cause band distortion, but does not reflect the molecular weight. Please use the result obtained using this marker as an index of the WB transfer efficiency. At least one blank lane is needed between the solution containing this marker and other solutions.

Fig. 2 Comparison of Prestained Markers



1, 2 : prestained marker (Company A) (3  $\mu$ L)  
 3, 4, 5 : WIDE-VIEW™ Prestained Protein Size Marker III (3  $\mu$ L)  
 6 : WIDE-VIEW™ Prestained Protein Size Marker III (5  $\mu$ L)  
 Blank lanes : 1 x sample buffer (5  $\mu$ L)  
 SuperSep Phos-tag™ (50  $\mu$ M), 12.5% (20 mA constant current)

### Phosphorylation reaction with coexisting ATP

Q. Does ATP in a phosphorylation reaction solution affect electrophoresis?

A. ATP has no particular effect at a concentration of 2.0 mM. The limit of use has not been investigated yet.

### Precast gel

Q. Can we use Phos-tag™ Acrylamide in an ordinary precast gel by adding it to sample solution?

A. No, you cannot. We have various kinds of precast Phos-tag™ gels called “SuperSep Phos-tag™” shown on page #27.

### Interpretation of multiple bands

Q. A band shift was observed in Phos-tag™ SDS-PAGE and multiple bands were detected. How can I decide whether the observation demonstrates phosphorylation or just degradation of the target protein?

A. Perform an ordinary SDS-PAGE (not using Phos-tag™) simultaneously to confirm that the target protein has not been degraded.

### DNA Separation using Phos-tag™

Q. Is Phos-tag™ applicable to separate DNA?

A. Refer to the following articles:

- A SNP genotyping method using phosphate-affinity polyacrylamide gel electrophoresis, *Analytical Biochemistry*, **361**, 294-298 (2007), E. Kinoshita, E. Kinoshita-Kikuta, and T. Koike (The phosphate group at DNA-terminal is efficiently captured by  $Zn^{2+}$ -Phos-tag™.)
- A mobility shift detection method for DNA methylation analysis using phosphate affinity polyacrylamide gel electrophoresis, *Analytical Biochemistry*, **378**, 102-104 (2008), E. Kinoshita-Kikuta, E. Kinoshita, and T. Koike

## ■ Phos-tag™ Biotin (Please see the page #27.)

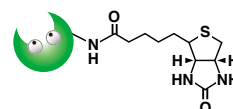
Q. What is the difference between BTL-104 and BTL-111?

A. BTL-104 and BTL-111 have linkers with different lengths.

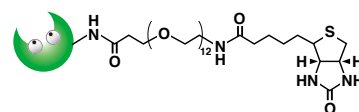
BTL-104 is recommendable as the first choice because of its high solubility. BTL-111 offers high sensitivity.

Q. What is the sensitivity level like?

A. It is at the nanogram level. Use a high-luminescence reagent such as ImmunoStar LD.



BTL-104  
MW:767



BTL-111  
MW:1,367

### Other reagents required

Q. Are there any reagents required other than the product?

A. Prepare a chemiluminescent reagent such as streptavidin-conjugated HRP solution and ImmunoStar Series. See "10" for ImmunoStar Series.

Q. For how many tests can Phos-tag™ Biotin be used?

A. Please refer to the following as a guide.

BTL-104 : 130~1300 tests

BTL-111 1 mM Aqueous Solution : 10~100 tests

Q. Can phosphorylated proteins be assayed?

A. You can do semi-quantitative assay based on the density of bands.

Q. Is it possible to determine the number of bound phosphate groups?

A. No, it isn't.

### Cell/tissue staining

Q. Is the product applicable to the staining of cells and tissues?

A. No. It is unsuitable for this application because it is removed by washing with methanol, etc.

Q. Can I strip Phos-tag™ Biotin?

A. Yes, you can. Mix it with a solution containing 62.5 mM of Tris-HCl (pH 6.8), 2% (w/v) of SDS, and 0.1 M of 2-mercaptoethanol and shake the mixture for 15 minutes.

Then, wash the mixture with 1×TBS-T three times for 10 minutes each time.

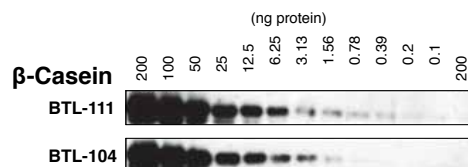
For further details, please contact us.

Q. What kind of membrane is recommended?

A. We recommend PVDF membranes.

Q. Does the use of Phos-tag™ Biotin require blocking?

A. No, it doesn't. Blocking causes the sensitivity to drop.



## ■ Phos-tag™ Mass Analytical Kit (Please see the page #27.)

Q. For how many tests can Phos-tag™ Mass Analytical kit be used?

A. More than 1,000 tests when 5 μL is used per test.

Q. How can I know which one of Phos-tag™ MS-101L, Phos-tag™ MS-101H, and Phos-tag™ MS-101N is appropriate?

A. Phos-tag™ 101N contains naturally occurring zinc species, 101L contains <sup>64</sup>Zn, and 101H contains <sup>68</sup>Zn.

Please refer to the following guidance: ① For exploring the conditions: Use 101N.

② Many isotopes contained in it make the spectrum complicated. Verification of the presence of phosphate groups: Use 101L and 101H.

These reagents contain zinc with a mass number of 64 and 68, respectively.

Measurement of a single sample with these reagents therefore results in a difference in m/e of 8.



## Detection of non-phosphorylated molecules

Q. Why are the peaks of non-phosphorylated molecules not detected?

A. Because the ionization efficiency differs considerably between phosphorylated molecules and nonphosphorylated molecules. The sample solution using Phos-tag™ is suited to the method using a buffer of pH 6 to 8 and a weak-acidic phenol matrix (such as THAP) and weak-basic HAMAN.

In the peptide analysis in the general positive mode, on the other hand, acidic sample solutions and acidic matrix are used.

Therefore, the ionization efficiency of the phosphorylated molecule-Phos-tag™ complex increases dramatically whereas that of the non-phosphorylated molecules becomes extremely low.

Q. I would like to measure a sample isolated by Phos-tag™ SDS-PAGE. Is it necessary to remove Phos-tag™ before in-gel digestion?

A. No, it isn't. Please follow the usual procedure for in-gel digestion after SDS-PAGE.

Q. Can it be also used for ESI mass spectrometry?

A. Yes, it can. Please refer to the following publication, which reports an example of ESI-MS analysis in which Phos-tag™ MS-101N was used as probe. A neutral solution should be used because analysis in an acidic solution causes Phos-tag™ to be detached.

Reference: *Anal. Chem.* (2008), **80**, 2531-2538 (MS-101N ESI-MS)

## Phos-tag™ Agarose (Please refer to the page #28.)

Q. Can samples purified by using Phos-tag™ Agarose be directly applied to SDS-PAGE?

A. No, they can't. The elution buffer recommended in the protocol contains a high concentration of salt and may cause the bands to be distorted. Please use the SDS-PAGE sample buffer as elution buffer.

Q. Is Phos-tag™ Agarose reusable?

A. We do not recommend it.

Q. Does Phos-tag™ Agarose have any advantages over IMAC?

A. Phos-tag™ Agarose allows experimental processes under physiological conditions (pH 7.5), and since it does not use reductants or surfactants, it can refine phosphorylated proteins in their native shape.

Also, the purified proteins can be used in processes such as mass spectrometry and Western blotting.

## Purification of His-tag proteins

Q. Is the product applicable to purification of His-tag phosphorylated proteins?

A. As His-tag exhibits weak affinity to Zn<sup>2+</sup>, use a tag of other types such as GST, if possible.

It is assumed that Zn<sup>2+</sup> shows a higher affinity to Phos-tag™ than to His-tag since isolation of His-tag protein by Zn<sup>2+</sup>-Phos-tag™ SDS-PAGE has been reported although no result of purification of His-tag protein with Phos-tag™ Agarose has been confirmed.

Q. What reagents are suitable and unsuitable for use in the sample preparation?

A. Please refer to the table below.

Category	Reagent	Suitability	Allowable concentrations
Reducing agents	DTT	○	≤ 0.1 M
Denaturing agents	Urea	○	Using it at 8M has no negative effect.
Surfactants (anionic)	SDS	○	Using it at ≥ 0.5% affects the binding process.
	Sodium deoxycholate	○	Using it at ≥ 0.25% affects the binding process.
Surfactants (nonionic)	Nonidet P40	○	≤ 1 %
	Tween 20	○	≤ 1 %
Surfactants (amphoteric)	CHAPS	○	≤ 0.2 %
Phosphate derivatives	β-Glycerophosphate	×	Do not use it.
	Pyrophosphate	×	Do not use it.
Chelating agents	EDTA	×	Do not use it.

# 8. SuperSep Phos-tag™

SuperSep Phos-tag™ is a precast gel, which can be immediately used after opening the package. It contains zinc as a supplemental metal. It has excellent storage stability by its neutral gel buffer and ZnCl<sub>2</sub>. Sharp bands can be obtained.

- Features**
- Ready-to-use
  - Safety due to precast gel
  - Long-term stability (Stable for 9 months)
  - Almost the same basic mechanism as normal SDS-PAGE
  - High reproducibility



SuperSep Phos-tag™

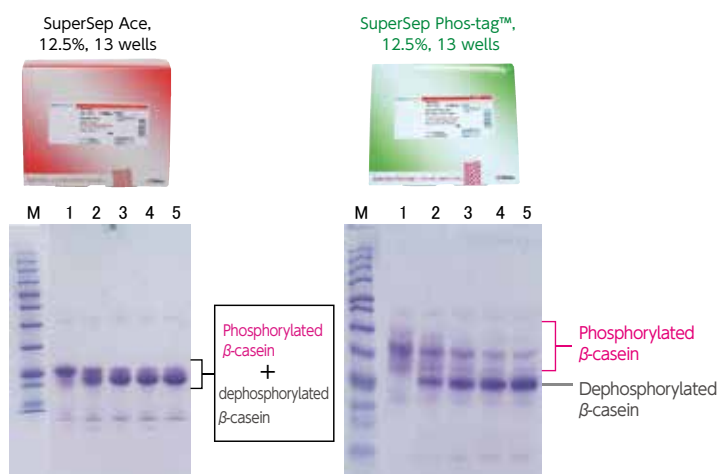
Plate Size	100 x 100 x 3 (mm)	ZnCl <sub>2</sub> conc.	100 μmol/L	
Gel Size	90 x 85 x 1 (mm)	Well volume	30 μL	25 μL
Well number	13	17	Storage Condition	
Phos-tag™ conc.	50 μmol/L		Keep at 2~10°C	

## ATTENTION

- Use of a normal prestained marker will distort the bands. Use of WIDE-VIEW™ Prestained Protein Size Marker III (Wako Cat. No. 230-02461) is recommended. Please refer to "Phos-tag™ Acrylamide - Molecular Marker" in "7. FAQ." in the page #23.
- Before using this product, check a sample for migration pattern problems with "SuperSep Ace" or other normal SDS-PAGE.
- When performing Western blotting, execute an EDTA process before transfer. For details, refer to "4. Troubleshooting."

## Application ~ Dephosphorylation over time of β-casein ~

Refer to the page #20 on WB data.



[Buffer]  
Tris-Glycine-SDS electrophoresis buffer

[Sample]

M: WIDE-VIEW™ Prestained Protein Size Marker III (Wako Cat. No. 230-02461)

- 1 : 0 min. β-casein (with AP treatment)
- 2 : 15 min. β-casein (with AP treatment)
- 3 : 30 min. β-casein (with AP treatment)
- 4 : 45 min. β-casein (with AP treatment)
- 5 : 60 min. β-casein (with AP treatment)

[Condition]

Constant current 35 mA for 60 min.

[Staining]

Quick-CBB Staining (Wako Cat. No. 299-50101)

[Destaining]

Deionized water with microwave

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

## Product List

### (1) for Bio-Rad's Electrophoresis Tank NEW

Product Name	Pkg. Size	Wako Cat. No.	Electrophoresis Tank
SuperSep Phos-tag™ (50 μmol/L), 7.5%, 17 wells 83×100×3.9 mm	5 gels	198-17981	Mini-PROTEAN® Tetra Cell (Bio-Rad Laboratories, Inc.)
SuperSep Phos-tag™ (50 μmol/L), 12.5%, 17 wells 83×100×3.9 mm		195-17991	


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

### (2) for Life Technologies' Electrophoresis Tank NEW

Product Name	Pkg. Size	Wako Cat. No.	Electrophoresis Tank
SuperSep Phos-tag™ (50 μmol/L), 7.5%, 17 wells 100×100×6.6 mm	5 gels	192-18001	XCell SureLock™ Mini-Cell (Life Technologies, Inc)
SuperSep Phos-tag™ (50 μmol/L), 12.5%, 17 wells 100×100×6.6 mm		199-18011	

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

### (3) for Wako's Electrophoresis Tank

Product Name	Wako Cat. No.	Pkg. Size	Electrophoresis Tank
SuperSep Phos-tag™ (50 μmol/L), 6%, 13 wells	192-17401	5 gels	EasySeparator 
SuperSep Phos-tag™ (50 μmol/L), 6%, 17 wells	199-17391		
SuperSep Phos-tag™ (50 μmol/L), 7.5%, 13 wells	195-17371		
SuperSep Phos-tag™ (50 μmol/L), 7.5%, 17 wells	192-17381		
SuperSep Phos-tag™ (50 μmol/L), 10%, 13 wells	193-16711		
SuperSep Phos-tag™ (50 μmol/L), 10%, 17 wells	190-16721		
SuperSep Phos-tag™ (50 μmol/L), 12.5%, 13 wells	195-16391		
SuperSep Phos-tag™ (50 μmol/L), 12.5%, 17 wells	193-16571		
SuperSep Phos-tag™ (50 μmol/L), 15%, 13 wells	193-16691		
SuperSep Phos-tag™ (50 μmol/L), 15%, 17 wells	196-16701		

These SuperSep Phos-tag™ precast gels are designed for the 'EasySeparator' tank.

Product Name	Wako Cat. No.	Pkg. Size	Electrophoresis Tank
EasySeparator™	058-07681	1 unit	—



# 9. Phos-tag™ Series

Product Name	Pkg. Size	Wako Cat. No.	Maker's code No.	Applications
Phos-tag™ Biotin BTL-111 1 mM Aqueous Solution (Ref.1)	0.1 mL	308-97201	BTL-111S1	BTL-111 offers higher sensitivity than BTL-104.
Phos-tag™ Biotin BTL-104	10 mg	301-93531	BTL-104	Specific detection without any anti-phosphorylated antibodies on Western blot. Detection is possible regardless of the type of phosphorylated amino acid.
Phos-tag™ Mass Analytical Kit	1 Kit	305-93551	MS-101KIT	Analysis by MALDI-TOF/Mass
Phos-tag™ Agarose	0.5 mL	302-93561	AG-501	Enrichment, separation and purification of phosphorylated proteins using column chromatography
	3 mL	308-93563	AG-503	
Phos-tag™ Tip	8tips	387-07321	AG2-103	

## Phos-tag™ Biotin — Detection of phosphoprotein by Western blotting —

NARD institute, Ltd.

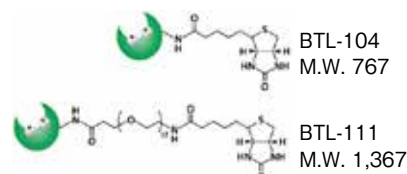
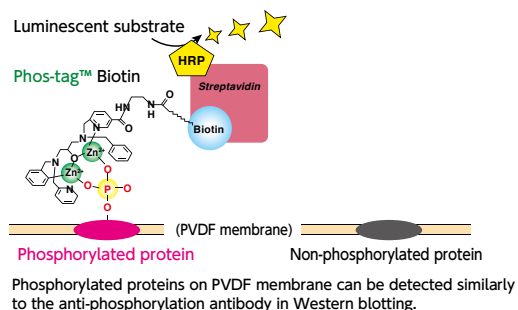
This biotin-bound Phos-tag™ is used for the detection of phosphoprotein by Western blotting.

- Features**
- Recommended when anti-phosphorylated antibodies are not available regardless of the type of phosphorylation.
  - No special reagent or device is necessary.

It can be conveniently used even if target anti-phosphorylated Thr/Ser antibody is not available !

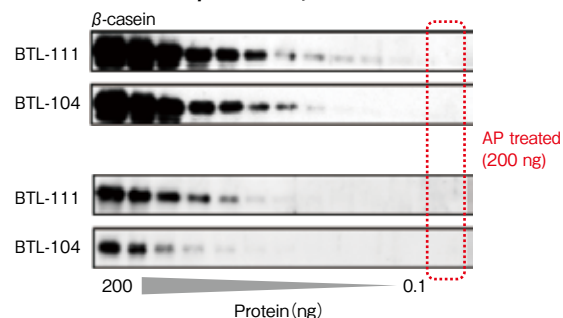
※ BTL-104, and BTL-111 have linkers with different lengths. BTL-111 offers higher sensitivity.

### Schematic of western blotting using Phos-tag™ Biotin



### Application

~Detection of  $\beta$ -casein, Ovalbumin~



## Phos-tag™ Mass Analytical Kit

NARD institute, Ltd.

– Detection Sensitivity of MALDI-TOF/Mass is improved –

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 molecules usually difficult to detect can be detected with improved sensitivity. Three types of reagents contain different types of zinc.

- Feature**
- Detection sensitivity of phosphorylated molecule is improved.
  - Non-phosphorylated molecules are not detected.
  - Applicable to phosphorylated molecules other than phosphorylated peptides.

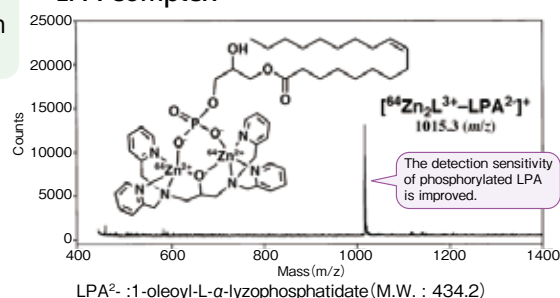
### [Kit Contents]

- Phos-tag™ MS-101L ..... 5 mg  
( $[C_{27}H_{29}N_6O^{64}Zn]^{3+}$  MW : 581.4)
- Phos-tag™ MS-101H ..... 5 mg  
( $[C_{27}H_{29}N_6O^{65}Zn]^{3+}$  MW : 589.4)
- Phos-tag™ MS-101N ..... 10 mg  
( $[C_{27}H_{29}N_6OZn]^{3+}$  MW : 584.3)

The detection sensitivity of phosphorylated LPA is improved.

### Application

~Detection of Phos-tag™-phosphorylated LPA complex~

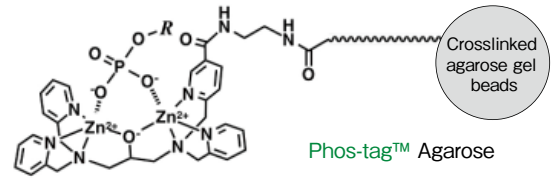


## Phos-tag™ Agarose

– Purification of phosphorylated proteins by affinity chromatography –

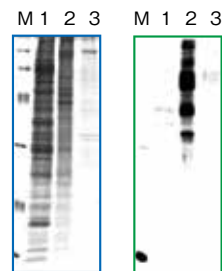
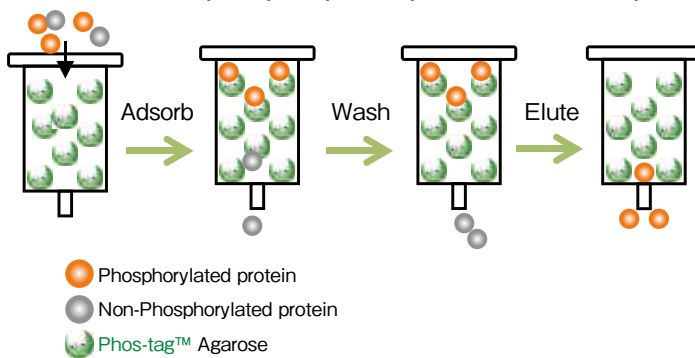
Fill Phos-tag™ Agarose in a column for use. It can be used for separation, purification and concentration of phosphorylated proteins. Because it is free of surfactants and reducing agents, phosphorylated proteins can be obtained in a condition similar to *in vivo*.

- Features**
- Phosphorylated proteins can be purified within 1 hour.
  - The proteins can be trapped in physiological condition (pH 7.5).
  - Purified without reducing agent and surfactant.



## Application

~Purification of phosphorylated proteins in A431 lysate~



M : Molecular Marker  
Lane 1 : Non adsorbed fraction  
Lane 2 : Adsorbed fraction  
Lane 3 : Column rinsing fraction  
(Left) Fluorescence staining  
(right) Western blotting with anti-phosphorylated Tyr

Lysate was applied to a column filled with Phos-tag™ Agarose. Phosphorylated proteins were concentrated in the adsorbed fraction.

NARD institute, ltd.

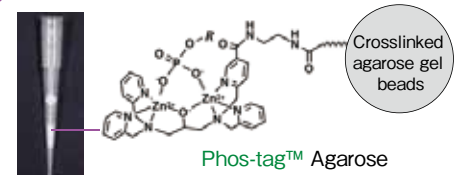
## Phos-tag™ Tip

– Ready-to-use Phos-tag™ tip for the concentration of phosphorylated peptides –

- Features**
- The operation time is less than 30 minutes.
  - High recovery rate
  - No expensive device required

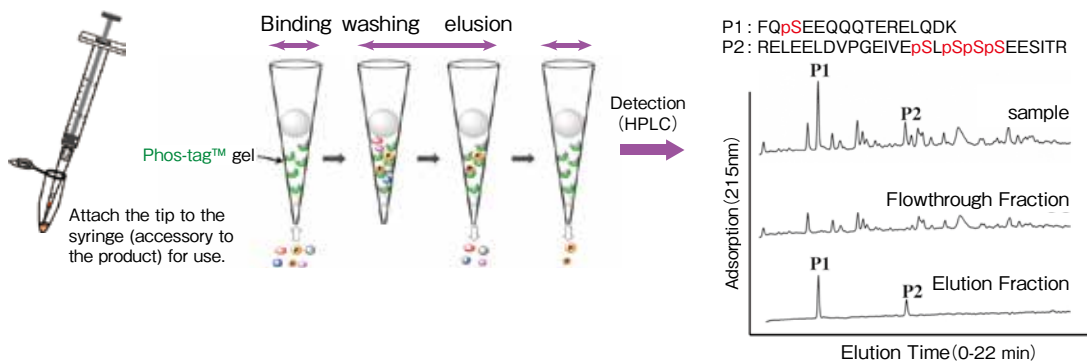


Phos-tag™ Tip



## Application

~Isolation of substances digested with 6 nmol β-casein trypsin~



# 10. Related Products (Electrophoresis/Western Blotting)

## Reagents for Phos-tag™ SDS-PAGE gel preparation

Product Name	Pkg. Size	Wako Cat. No.	Use Application
30 w/v% Acrylamide / Bis Mixed solution(29:1)	500 mL	015-25635	Ready-to-use "Solution A". 30%T, 3.3%C
Agarose H (High-strength type)	1 g	315-01203	High-strength Agarose has high strength even in a low-agarose environment and is suitable for electrophoretic migration of large nucleic acid fragments. It can be used in a concentration range of 0.2 - 1% and a separation range of 1 - 200 kbp.
	10 g	319-01201	
	25 g	317-01202	
10% SDS Solution	100 mL	311-90271	Ready-to-Use "Solution D"
	500 mL	313-90275	
Manganese(II) Chloride Tetrahydrate, 99.0+ % (Titration)	25 g	134-15302	for Molecular Biology Please use for preparation of "Solution F"
	100 g	136-15301	
Zinc Chloride	25 g	268-01902	for Molecular Biology. Please use for preparation of "Solution M"
Bis-Tris	100 g	345-04741	Please use for preparation of "Solution N"
Sodium Hydrogensulfite (JIS Special Grade)	25 g	196-01372	Please use for preparation of "Solution O"
	100 g	198-01371	
Sodium Hydrogensulfite (For Molecular Biology)	100 g	190-16461	
MOPS	100 g	345-01804	Please use for preparation of "Solution P"
	250 g	341-01801	
MOPS (For Molecular Biology)	100 g	341-08241	
	500 g	343-08245	
Separating Gel Buffer Solution (x4)	250 mL	192-11041	Ready-to-Use mixed solution of "Sol. B" and "Sol. D" for preparation of Resolving Gel. Contains SDS.
Stacking Gel Buffer Solution (x4)	250 mL	199-11051	Ready-to-Use mixed solution of "Sol. C" and "Sol. D" for preparation of Stacking Gel. Contains SDS.
<i>N,N,N',N'</i> -Tetramethylethylenediamine (TEMED)	25 mL	205-06313	for Electrophoresis
10 w/v% Ammonium Peroxodisulfate Solution (Ammonium Persulfate Solution)	25 mL	019-15922	Ready-to-Use "Solution G"

## Premixed Buffers

Product Name	Pkg. Size	Wako Cat. No.	Use Application
Running Buffer Solution (x10)	1 L	184-01291	Ready-to-Use concentrated "Solution H"
SDS-PAGE 10x Running Buffer	1 L	312-90321	Ready-to-Use concentrated "Solution H"
	5 L	318-90323	
SDS-PAGE Buffer, pH 8.5	5 L	192-16801	Ready-to-Use "Solution H", 1 x buffer.
Tricine Running Buffer Solution (x10)	1 L	200-17071	Composition: 0.5 M Tris / 0.5 M Tricine / 1% SDS
Sample Buffer Solution (2ME+) (x4)	25 mL	191-13272	Sample buffer for Laemmli SDS-PAGE containing 2-mercaptoethanol
Sample Buffer Solution (2ME+) (x2)	25 mL	196-11022	
Sample Buffer Solution with 3-Mercapto-1,2-propanediol (x2)	25 mL	199-16132	Laemmli Sample Buffer containing 3-mercapto-1,2-propanediol (non-hazardous chemical) as substitute for 2-ME
Sample Buffer Solution with 3-Mercapto-1,2-propanediol (x2)	25 mL	196-16142	
AquaBlot™ 10x Tris-Glycine-SDS Transfer Buffer	1 L	019-25111	Please use it to transfer to a membrane. Containing 0.05% SDS

## Enzyme for Dephosphorylation

Product Name	Pkg. Size	Wako Cat. No.	Use Application
Alkaline Phosphatase (for Biochemistry)	50 U	018-10693	Applicable to dephosphorylation of proteins
	100 U	012-10691	

## Reagents for Staining

Product Name	Pkg. Size	Wako Cat. No.	Use Application
Quick CBB Plus	250 mL	174-00553	Ready-to-Use Sol. K. Fixing and destaining procedure are not required. No organic solvents are necessary. Protein bands are stained in 10 minutes.
	1 L	178-00551	
Quick-CBB · Staining solution A: 1L × 1 · Staining solution B: 1L × 1	2 L	299-50101	By mixing staining solution A and B, ready-to-Use Sol. K
Silver Stain MS Kit	20 tests	299-58901	Modified proteins by glycosilation and / or phosphorylation can be detected sub-nanogram level on the electrophoretic gel.
Silver Stain Kit <i>Wako</i>	for 10 gels	299-13841	50~100 times more sensitive than CBB method.
Silver Stain II Kit <i>Wako</i>	for 10 gels	291-50301	This kit contains Stopper, which can be adjusted the staining strength.
Negative Gel Stain MS Kit	20 tests	293-57701	Applicable to mass analysis and Western blot

## Protein Size Marker

Product Name	Pkg. Size	Wako Cat. No.	Use Application
WIDE-VIEW™ Prestained Protein Size Marker III	25 µL	236-02463	A recommendable prestained marker used with Phos-tag™ SDS-PAGE because obtained bands are less distorted.
	500 µL	230-02461	
	500 µL × 3	234-02464	

## Positive Control (for confirmation of separation capacity of Phos-tag™ SDS-PAGE)

Product Name	Pkg. Size	Wako Cat. No.	Use Application
α-Casein, from Bovine Milk, Dephosphorylated	1 mg	038-23221	Mixture of phosphorylated and dephosphorylated α-casein
	10 mg	034-23223	

## Electrophoresis Apparatus • Precast Gels

Product Name	Pkg. Size	Wako Cat. No.	Use Application
EasySeparator	1 unit	058-07681	An electrophoresis tank for SuperSep precast polyacrylamide gels.
SuperSep Ace, 6%, 13 wells	10 gels	195-15171	Prior to use of SuperSep Phos-tag™ PAGE, please use these as sample confirmation. Expired in 9 months after manufacture
SuperSep Ace, 7.5%, 13 wells	10 gels	198-14941	
SuperSep Ace, 7.5%, 17 wells	10 gels	191-14931	
SuperSep Ace, 10%, 13 wells	10 gels	195-14951	
SuperSep Ace, 10%, 17 wells	10 gels	192-14961	
SuperSep Ace, 12.5%, 13 wells	10 gels	199-14971	
SuperSep Ace, 12.5%, 17 wells	10 gels	196-14981	
SuperSep Ace, 15%, 13 wells	10 gels	193-14991	
SuperSep Ace, 15%, 17 wells	10 gels	190-15001	

## Reagents for Western Blotting

Product Name	Pkg. Size	Wako Cat. No.	Use Application
ImmunoStar LD* · Luminescence solution A · Luminescence solution B	200 cm <sup>2</sup>	296-69901	Highly sensitive (femto gram level) immunoblotting, utilizing detection by enhanced chemiluminescence using a unique luminol derivative L-012 as a substrate. Not available for sale in the US and Europe.
	1,000 cm <sup>2</sup>	292-69903	
	2,000 cm <sup>2</sup>	290-69904	
ImmunoStar Zeta*	200 cm <sup>2</sup>	291-72401	Use for detection of proteins between the middle and low femto gram levels. Has stable, long-lasting luminescence signal.
	1,000 cm <sup>2</sup>	297-72403	
	2,000 cm <sup>2</sup>	295-72404	
ImmunoStar Basic*	200 cm <sup>2</sup>	295-75101	A cost-effective, stable and long-lasting product.
	2,000 cm <sup>2</sup>	291-75103	
	5,000 cm <sup>2</sup>	299-75104	
Immuno Enhancer	2 assays	294-68601	Ready-to-Use Immunoreaction Enhancer for Western blotting and ELISA
	10 assays	290-68603	
	40 assays	298-68604	

\* : Not available for sales in the US and Europe.



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