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## Infinite possibilities of Phos-tag™ SDS-PAGE

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<tr>
<th>in vitro</th>
<th>Expressed Proteins</th>
<th>Cells</th>
<th>in vivo</th>
<th>Animal Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinase assay</td>
<td>Determination of phosphorylated/ non-phosphorylated proteins</td>
<td>Analysis of phosphorylation of endogenous proteins</td>
<td>Analysis of genetically modified mice</td>
<td></td>
</tr>
<tr>
<td>Analysis of Kinase Analysis casasdes</td>
<td>Increment/Decrement of phosphorylation by stimulation</td>
<td>Requires little effort of preparation of phosphorylated antibodies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No Radioisotope Treatment

Ready for research of phosphorylation of various stages and various purposes!
1. Principle and Application

**Phos-tag™ is ...**

Phos-tag™ is a functional molecule that binds specifically phosphorylated ions. It is applicable for the specific separation of phosphorylated proteins (Phos-tag™ Acrylamide) as well as for the detection using western blot (Phos-tag™ Biotin), purification (Phos-tag™ Agarose), and MALDI-TOF/MS (Phos-tag™ Mass Analytical Kit).

**Basic Structure of Phos-tag™**

![Basic Structure of Phos-tag™](image)

M²⁺: Zinc ion or manganese ion
d Selectivity of binding of a phosphate ion (2-) is much higher than that of other anions.
d Stable complex is formed under physiological conditions (pH 5 to 8).

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

Phos-tag™ was developed by Department of Functional Molecular Science at Hiroshima University. [http://www.phos-tag.com/](http://www.phos-tag.com/)

**Principle**

Two metallic ions cooperate to bind a phosphate group.

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

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

**Application**  Time course of phosphorylation by using the Tyrosin Kinase Abl —

Phosphorylated tyrosine was prepared by GST binding protein of tyrosine kinase Abl and the substrate peptide (Abtlride) and separated with conventional SDS-PAGE and Phos-tag™ SDS-PAGE, respectively.

<table>
<thead>
<tr>
<th>Conventional SDS-PAGE (CBB staining)</th>
<th>Phos-tag™ SDS-PAGE (CBB staining)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinase Reaction Time</td>
<td>Kinase Reaction Time</td>
</tr>
<tr>
<td>0 1 5 10 20 30 60 (min.)</td>
<td>0 1 5 10 20 30 60 (min.)</td>
</tr>
<tr>
<td>Phosphorylated + Non-phosphorylated proteins</td>
<td>Phosphorylated proteins</td>
</tr>
<tr>
<td>One band = Inseparable</td>
<td>Non-phosphorylated proteins</td>
</tr>
<tr>
<td></td>
<td>2 bands = Separable</td>
</tr>
</tbody>
</table>
2. Phos-tag™ Acrylamide is…

**Phos-tag™ Acrylamide**

Phos-tag™ Acrylamide is an acrylamide-pendant Phos-tag™ molecule. Phos-tag™ SDS-PAGE, where a polyacrylamide gel containing Phos-tag™ is used, can be prepared by adding Phos-tag™ Acrylamide and MnCl₂ in Resolving Gel when preparing SDS-PAGE gels. During migration, the phosphorylated proteins with the phosphate group bound to the divalent metal ions in Phos-tag™. This decreases the migration speed and phosphorylated/non-phosphorylated proteins are separated. After separation, the gel can be utilized for western blotting and mass spectrometry. Various Phos-tag™ SDS-PAGE precast gels are also available. Please see 8. SuperSep Phos-tag™ on the page #19 for the details.

By simply adding Phos-tag™ Acrylamide and MnCl₂ to an acrylamide solution when preparing SDS-PAGE gels, Phosphorylated proteins are easily separable.

![Time-course analysis of β-casein using Phos-tag™ SDS-PAGE](image)

**Development from Phos-tag™ SDS-PAGE**

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

### Western blotting

- Easily recognizable phosphorylation of your target proteins
- Simultaneous detection of phosphorylated/non-phosphorylated proteins with a general antibody by their band shift differences.
- No need to prepare the phosphorylated antibody.
- Applicable to analysis of phosphorylation of endogenous proteins.
- Application Data → See the page #13.

<table>
<thead>
<tr>
<th>MC</th>
<th>ATP</th>
<th>Pos 1</th>
<th>Pos 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* MC: Microcystin (a phosphatase inhibitor)

(Laemmli method)

(Phos-tag™ SDS-PAGE)

Sample: Rat brain extract
Detection: Anti pS5
Lane 1: Brain extract before incubation.
Lane 2-5: Incubate with (+) and without (-) MC or ATP

Data was provided by Tomohisa Hosokawa at Brain Science Institute, RIKEN (Japan)

### Mass Analysis

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

### 2D Electrophoresis

Phosphorylated forms with the same isoelectric points (same number of phosphorylation sites) can be separated. Application Data → See the page #12

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No. (Nard Product #)</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phos-tag™ Acrylamide 5 mM Aqueous Solution</td>
<td>0.3 mL (0.9 mg)</td>
<td>304-93526 (AAL-107S1)</td>
<td>Separation of phosphorylated and non-phosphorylated proteins using SDS-PAGE with Phos-tag™ Acrylamide</td>
</tr>
<tr>
<td>Phos-tag™ Acrylamide</td>
<td>2 mg</td>
<td>300-93523 (AAL-107M)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg</td>
<td>304-93521 (AAL-107)</td>
<td></td>
</tr>
</tbody>
</table>
3. Protocol

[I] Mn$^{2+}$-Phos-tag™ SDS-PAGE

Note: Always prepare the gel just before use.

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

Acrylamide Solution

- **Sol. A**: 30 w/v% Acrylamide 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

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

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
  - 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
  - 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)

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

- **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)
  - The oily product, Phos-tag™ AAL-107 is provided in a small plastic tube and 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.
  - [Storage] Wrap the tube with aluminum foil.
  - Keep the soln. in a 2-mL microtube at 4°C in the dark.
**Sol. F**: 10 mmol/L MnCl₂ Solution

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

# MnCl₂(H₂O)₆ (MW: 198) …………… 0.10 g
# Distilled water …………………… 50 mL

Note: Do not use other anion salts such as Mn(NO₃)₂ or Mn(CH₃COO)₂. White precipitates (Mn(OH)₄) 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 Mn²⁺ will deteriorate.

**APS Solution**

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

# (NH₄)₂S₂O₈ (MW: 228) …………… 10 mg
# Distilled water …………………… 0.10 mL

⇒ Prepared sol. G can be stored for long period at -20°C by aliquot in an appropriate amount.

**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

⇒ Prepare the 500 mL 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.

**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

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

[Storage] Keep at -20°C.

Usage of Soln. I: Please see the "8. Sample Preparation" (page #9).

**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 Sln.**

**Applicable to silver staining and fluorescent staining.**

**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

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

**Washing and Destaining Sln.**

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

# methanol …………………….. 0.25 L
# acetic acid …………………… 0.10 L
# Distilled water ………………… 0.65 L
Resolving Gel Solution (0.375 mol/L Tris, 0.1 mmol/L MnCl₂, 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 Solution

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

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

# Sol. F: 10 mmol/L MnCl₂ Solution

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

# TEMED (tetramethylethylenediamine)

# Distilled Water

~Dereate with string for 2 minutes.~

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

Note

Please optimize concentration of Sol. E (Phos-tag™) and Sol. A (Acrylamide).
Refer to 5. Optimization of Phos-tag™ PAGE Condition (page #11).

### Reference

Examples of preparation of 10 mL of resolving gel solution

<table>
<thead>
<tr>
<th>Phos-tag™ Acrylamide conc.</th>
<th>20 µM</th>
<th>50 µM</th>
<th>100 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide conc.</td>
<td>12%</td>
<td>10%</td>
<td>8%</td>
</tr>
<tr>
<td>Sol. A (mL)</td>
<td>4</td>
<td>3.33</td>
<td>2.67</td>
</tr>
<tr>
<td>Sol. B (mL)</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Sol. E (mL)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Sol. F (mL)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Sol. D (mL)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>TEMED (mL)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Distilled water (mL)</td>
<td>3.2</td>
<td>3.87</td>
<td>4.53</td>
</tr>
<tr>
<td>Sol. G</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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 Solution

# Sol. C: 0.50 mol/L Tris/HCl Solution, pH 6.8

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

# TEMED (tetramethylethylenediamine)

# Distilled Water

~Dereate with string for 2 minutes.~

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

Note

Please optimize concentration of Sol. E (Phos-tag™) and Sol. A (Acrylamide).
Refer to 5. Optimization of Phos-tag™ PAGE Condition (page #11).
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 MnCl₂, 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 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 MnCl₂ Solution ...................................................... 0.04 mL *1)
- **Sol. D**: 10% (w/v) SDS Solution ......................................................... 0.10 mL
- TEMED (tetramethylethlenediamine) .................................................... 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)

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

*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.

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 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 (tetramethylethlenediamine) .................................................... 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.
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.

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, refer to Sec. [Ⅱ] below after electrophoretic migration has occurred.

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 photograph of the gel.

[Ⅱ] Tips for Western blotting of Phos-tag™ SDS-PAGE gels -important-

After electrophoresis, an additional procedure, i.e., elimination of the manganese ion (Mn^{2+}) from the gel using chelating agent (EDTA), is necessary before electroblotting. This procedure increases the transfer efficiency of the phosphorylated and non-phosphorylated proteins onto a PVDF membrane.

1) Just after electrophoresis, the gel is soaked in a general transfer buffer containing 1～10 mmol/L EDTA for a minimum of 10 minutes with gentle agitation. (for 10 minutes x 1～3 times).
   ※ Change the temperature and treatment time with transfer EDTA-buffer according to the gel thickness, etc. (eg: 1.5 mm thick: 20 minute treatment x twice).
   ※ Besides transfer buffer, 1 x Running buffer can be also used .

2) Next, the gel is soaked in a general transfer buffer without EDTA for 10 minutes with gentle agitation (for 10 min. x 1 time).
   ※ a wet-tank method is strongly recommended for effective protein transfer from the Mn^{2+}-Phos-tag™ acrylamide gel to the PVDF membrane. (The semi-dry method can also be used.)
   ※ The blotting conditions, such as time and temperature, must be optimized for your phosphorylated target protein in the Phos-tag™ gel.

[Ⅲ] Tips for Mass Spectrometry of Phos-tag™ SDS-PAGE

No special procedures such as EDTA treatment are necessary.
4. Trouble Shooting

Distortion of bands
The most common complaint for Phos-tag™ SDS-PAGE is “Distortion of bands. Especially, make sure not to contain EDTA in your samples.”

1. Prestained marker: Not only the lane of the marker but also the sample lane may be affected due to a difference in the salt concentration among lanes.
   ⇒ Prestained markers should not be used. (See the right figure.) We recommend to use alkaline phosphatase-treated sample or recombinant protein of your target one as the negative control of phosphorylation instead of using prestained markers.

2. Acidic sample: Bands may be distorted. If the solution is a yellow to orange color even after loading sample buffer, add Tris buffer until it is neutral (violet).

3. EDTA (Mn²⁺ is chelated), vanadic acid, inorganic salts, surfactants, etc., cause distortion or tailing of bands. ⇒ Desalinate by precipitation with TCA or dialysis.

4. Blank lanes: Blank lanes may cause distortion. ⇒ Load the same amount of 1 x sample buffer in blank lanes.

5. Vanadic acid: Competitive binding with phosphoric acid may cause distortion. ⇒ Use a different phosphatase inhibitor or remove vanadic acid by precipitation with TCA or dialysis.

Adding MnCl₂ to the sample to be applied (eg: 1 mM final concentration) may improve results. If the sample contains an EDTA residue, it is because the added Mn²⁺ is chelated instead of the Mn²⁺ contained in the gel.

Low Resolution

1. Rise of the molar ratio of MnCl₂ to Phos-tag™ Acrylamide may improve the resolution. (eg: 1 : 4)

2. Adopting Tris-Tricine Buffer as Running Buffer may improve the resolution.

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.”

Trouble with Transfer onto the membrane

1. Treatment with EDTA may be insufficient. Increase the treatment time, frequently exchange the buffer containing EDTA (eg: 20 min. twice), or fully agitate during EDTA treatment.

2. To intensify the electric current may improve efficiency (eg: 200 mA)

3. Stainings other than negative staining such as CBB staining may diminish the transfer efficiency.

4. Using a low-concentration gel will improve the transfer efficiency.

5. Take steps such as using a thick gel and increase the sample application amount.

6. Transfer buffer containing SDS may improve the transfer efficiency. When transferring onto a membrane, immediately add the SDS solution to the transfer buffer to prevent sudsing (tank method), or between the EDTA process and transfer, immerse the gel in a transfer buffer containing SDS and shake slowly (eg: for 10 min. x 1 time). Try SDS concentrations of 0.05 ~ 0.20%.

Higher background during staining

Stain after eliminating metal ions in the gel by EDTA treatment.

Mobility shift due to protein degradation, not induced by phosphorylation

Carry out SDS-PAGE as usual (containing 0 µM of Phos-tag™) and verify that no mobility shift occurs.
5. Optimization of Phos-tag™ PAGE Condition

To obtain a high quality result using Phos-tag™ 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™ 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 optimum for Phos-tag™ SDS-PAGE.

![Indication of optimum concentration](image)

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 is a 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-methylenebisacylamide content (eg: 5% acrylamide [24:1]).

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

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

[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 μM. However, a higher concentration, eg: 100 μM, 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 at Science Research Center, Kochi University)

Higher concentration of Phos-tag™ leads to higher separation capacity?

In general, a higher concentration leads to higher separation capacity. (Compare the samples of 50 μM and 100 μM of Mn²⁺-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 μM and 150 μM of ovalbumin of the right figure.)

**Distance between two bands**
- a-casein: 50 μM < 100 μM
- ovalbumin: 50 μM > 150 μM

Please adjust the optimum concentration for every target protein.

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6. Application Data and References

Application Data 1

Impressions of users of Phos-tag™ Acrylamide are introduced herein. We received data and comments from Dr. Tadayuki Ogawa of the University of Tokyo. In addition, we received data applied to two-dimensional electrophoretic migration from Dr. Yayoi Kimura of Yokohama City University, as well as Western blotting applied data from Dr. Yasunori Sugiya of Kochi University and Dr. Tomohisa 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 of proteins phosphorylated using Phos-tag™ SDS-PAGE and non-phosphorylated proteins

The kinase-reacted phosphorylated proteins in (2) - (5) show clear differences compared with the non-phosphorylated protein in (1). Data such as the quantitative ratio of phosphorylated and non-phosphorylated proteins, degree of phosphorylation, and population distribution can be readily obtained from band shifts and density.

(Source: Graduate School of Medicine, the University of Tokyo)

Application Data 2

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

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

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

Data published in:

Data provided by: Dr. Y. Kimura and Dr. H. Hirano, Yokohama City University and O. Ohara, RCAI, RIKEN.

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(revised in March, 2013)---
### Application Data 3

**Determining fraction containing kinase for phosphorylating Dnmt1**

Dnmt1: DNA methyltransferase

![Western blot](image)

- 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:


Data provided by: Dr. Y. Sugiyama, Laboratory of Molecular Biology, Science Research Center, Kochi University and Dr. I. Kameshita, Department of Life Science, Faculty of Agriculture, Kagawa University.

### Application 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)

![Western blot](image)

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.


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

**Regarding Phos-tag™ reagents:**


**Application using Phos-tag™ reagents**


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

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**Published papers related to Phos-tag™ by HighWire Search**

![Published papers graph](image_url)
Phos-tag™ Acrylamide

**Determination**

Q. Can phosphorylated proteins be assayed?
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. How large (kDa) can a protein be separated using this product?
A. A phosphorylated protein of 350 kDa has actually been separated with 20 µM Phos-tag™, 3% acrylamide and 0.5% agarose*.
   * Agarose was added to strengthen the gel.

Q. How can the resolution be improved?
A. In general, a higher concentration of Phos-tag™ results in a higher resolution. However, increasing the concentration of Phos-tag™ also causes the overall migration speed of the protein to proportionally drop.
   Please refer to the page #11.

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

**Gel Strength**

Q. The gel is easily broken. What can I do for this?
A. A low concentration of acrylamide causes the gel to be soft.
   You can solve this problem by increasing the relative amount of methylenebisacrylamide to acrylamide (24:1), for example.

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

Q. How long can the prepared gel containing Phos-tag™ Acrylamide be stored?
A. 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 6 months by refrigeration under protection from light. The solutions seem to be storable for 1 year without any problem according to doctors who are using the product.
Phos-tag™ Acrylamide (continued)

Preparation of the reagent

Q. Does the concentration of Phos-tag™ influence the amount of ions required to be bound to one Phos-tag™ molecule (Fig. 1).

A. The molar ratio of Phos-tag™ acrylamide to Mn²⁺ should be 1:2; two Mn²⁺ 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.

Molecular marker

Q. What pre-stained markers can we use?

A. Using a pre-stained 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 transfer efficiency. At least one blank lane is needed between the solution containing this marker and other solutions.

Phosphorylation reaction with coexisting ATP

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

A. ATP had 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 a precast gel by adding it to sample solution?

A. No, you cannot. We have various kinds of precast gels called "SuperSep Phos-tag™" shown on the page #19.

Differentiation between degraded protein and phosphorylated Phos-tag™

Q. A mobility shift was observed in Phos-tag™ SDS-PAGE. How can I know whether it is a sign of phosphorylation or only telling me that the protein was broken down?

A. Please carry out a conventional SDS-PAGE (without Phos-tag™) and verify that your protein is intact.

DNA Separation using Phos-tag™

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

A. Refer to the following articles:

SuperSep Phos-tag™ (Please see the page #19.)

Q. Do you have lower-concentration polyacrylamide products?
A. Products with concentrations of 6%, 7.5% and 10% are currently being developed.

Q. Do you have products with other Phos-tag™ polyacrylamide concentrations?
A. Products with concentrations of 20 μM and 100 μM are currently being developed.

Q. Are there ways to improve separation capacity?
A. Using a Tris-Tricine buffer as the running buffer improves separation capacity.

Q. Are there any references?

Phos-tag™ Biotin (Please see the page #20.)

Q. What is the difference of BTL-104, BTL-105 and BTL-111?
A. 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 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.

Q. Do we need other reagents besides this product?
A. Prepare a Streptavidin-conjugated HRP solution.

Q. How many times can Phos-tag™ Biotin be used?
A. It depends on the frequency of use. Please refer to the following as a guide.
   BTL-104 : 130~1300 times
   BTL-105 : 113~1130 times
   BTL-111 1 mM Aqueous Solution : 10~100 times

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 binding phosphate groups?
A. No, it isn’t.

Q. Can I strip the antibodies of 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 #20.)**

Q. How many tests can Phos-tag™ Biotin 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 64Zn, and 101H contains 68Zn. Please refer to the following guidance. Exploration of conditions: Use 101N. 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 16.

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.

**Phos-tag™ Agarose (Please refer to the page #20)**

Q. Can samples purified 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 all 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.

Q. What reagents are suitable and unsuitable for use in the sample preparation?
A. Please refer to the table below.

<table>
<thead>
<tr>
<th>Category</th>
<th>Reagent</th>
<th>Suitability</th>
<th>Allowable concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing agents</td>
<td>DTT</td>
<td>○</td>
<td>≤ 0.1 M</td>
</tr>
<tr>
<td>Denaturing agents</td>
<td>Urea</td>
<td>○</td>
<td>Using it at 8M has no negative effect.</td>
</tr>
<tr>
<td>Surfactants</td>
<td>SDS</td>
<td>○</td>
<td>Using it at ≥ 0.5% affects the binding process.</td>
</tr>
<tr>
<td>(anionic)</td>
<td>Sodium deoxycholate</td>
<td>○</td>
<td>Using it at ≥ 0.25% affects the binding process.</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Nonidet P40</td>
<td>○</td>
<td>≤ 1 %</td>
</tr>
<tr>
<td>(nonionic)</td>
<td>Tween 20</td>
<td>○</td>
<td>≤ 1 %</td>
</tr>
<tr>
<td>Surfactants</td>
<td>CHAPS</td>
<td>○</td>
<td>≤ 0.2 %</td>
</tr>
<tr>
<td>(amphoteric)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate derivatives</td>
<td>β-Glycerophosphate</td>
<td>×</td>
<td>Do not use.</td>
</tr>
<tr>
<td></td>
<td>Pyrophosphate</td>
<td>×</td>
<td>Do not use.</td>
</tr>
<tr>
<td>Chelating agents</td>
<td>EDTA</td>
<td>△</td>
<td>Using it at a high concentration has a negative effect.</td>
</tr>
</tbody>
</table>
8. SuperSep Phos-tag™

SuperSep Phos-tag™ is a precast gel, which can be immediately used after opening the package, is added with 50 μmol/L of Phos-tag™ Acrylamide in advance. It contains zinc as a supplemental metal. It has excellent storage stability by its neutral gel buffer. Sharp bands can be obtained.

Features
- Ready-to-use
- Safety due to precast gel
- Long-term stability (Stable for 6 months)
- Almost the same basic mechanism as that of SDS-PAGE

<table>
<thead>
<tr>
<th>Plate Size</th>
<th>100 x 100 x 3 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel Size</td>
<td>90 x 85 x 1 (mm)</td>
</tr>
<tr>
<td>Well number</td>
<td>13, 17</td>
</tr>
<tr>
<td>Phos-tag™ conc.</td>
<td>50 μmol/L</td>
</tr>
<tr>
<td>Acrylamide conc.</td>
<td>12.5% or 15%</td>
</tr>
<tr>
<td>ZnCl₂ conc.</td>
<td>100 μmol/L</td>
</tr>
<tr>
<td>Well volume</td>
<td>30 μL, 25 μL</td>
</tr>
</tbody>
</table>

ATTENTION
- This product is a precast gel optimized in an “EasySeparator” tank.
- 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 “Sec. 7. FAQ.” on the page #16.
- 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 "Sec. 4. Troubleshooting."

Application ~ Dephosphorylation over time of β-casein ~

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Package Size</th>
<th>Wako Cat. No.</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SuperSep Ace, 12.5%, 13 wells</td>
<td>10 gels</td>
<td>196-1491</td>
<td>Keep at 2~10°C</td>
</tr>
<tr>
<td>SuperSep Phos-tag™, 12.5%, 13 wells</td>
<td>5 gels</td>
<td>193-16571</td>
<td>Keep at 2~10°C</td>
</tr>
<tr>
<td>SuperSep Phos-tag™ (50 μmol/L), 12.5%, 17 well</td>
<td>5 gels</td>
<td>193-16691</td>
<td>Keep at 2~10°C</td>
</tr>
<tr>
<td>SuperSep Phos-tag™ (50 μmol/L), 15%, 13 well</td>
<td>5 gels</td>
<td>196-16701</td>
<td>Keep at 2~10°C</td>
</tr>
<tr>
<td>SuperSep Phos-tag™ (50 μmol/L), 15%, 17 well</td>
<td>5 gels</td>
<td>196-167101</td>
<td>Keep at 2~10°C</td>
</tr>
</tbody>
</table>

Related Products
- SuperSep Ace, 12.5%, 13 well | 10 gels | 199-14971 | Keep at 2~10°C |
- SuperSep Ace, 12.5%, 17 well | 10 gels | 196-14981 | Keep at 2~10°C |
- SuperSep Ace, 15%, 13 well | 10 gels | 193-14991 | Keep at 2~10°C |
- SuperSep Ace, 15%, 17 well | 10 gels | 190-15001 | Keep at 2~10°C |
- EasySeparator (an electrophoresis tank for SuperSep) | 1 unit | 058-07681 | RT |
- Wide-View Prestained Protein Size Marker III (11~245 kDa) | 500 μL (for 200 tests) | 230-02461 | Keep at -20°C |
9. Phos-tag™ Series

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No.</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phos-tag™ Biotin BTL-104</td>
<td>10 mg</td>
<td>301-93531</td>
<td>Specific detection without any anti-phosphorylated antibodies on Western blot. Detection is possible regardless of type of phosphorylated amino acid.</td>
</tr>
<tr>
<td>Phos-tag™ Biotin BTL-105</td>
<td>10 mg</td>
<td>308-83541</td>
<td>BTL-111 offers higher sensitivity than BTL-104.</td>
</tr>
<tr>
<td>Phos-tag™ Biotin BTL-111</td>
<td>0.1 mL</td>
<td>308-97201</td>
<td>Analysis by MALDI-TOF/Mass</td>
</tr>
<tr>
<td>Phos-tag™ Mass Analytical Kit</td>
<td>1 Kit</td>
<td>305-93551</td>
<td>Enrichment, separation and purification of phosphorylated proteins using column chromatography</td>
</tr>
<tr>
<td>Phos-tag™ Agarose</td>
<td>0.5 mL</td>
<td>302-93561</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 mL</td>
<td>308-93563</td>
<td></td>
</tr>
</tbody>
</table>

**Phos-tag™ Biotin** — Detection of phosphoprotein for Western blotting —

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

*Features*
- All phosphoprotein can be detected.
- Procedures of experiment are similar to those in ordinary Western blotting.

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

* 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.

*Reference of BTL-111*

1) Highly sensitive detection of protein phosphorylation by using improved Phos-tag Biotin, **Proteomics**, 12(7), 932-7 (2012), Kinoshita E, Kinoshita-Kikuta E, Sugiyama Y, Ozeki T, Koike T.

**Phos-tag™ Mass Analytical Kit**

—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 molecule usually difficult to detect can be detected with improved sensitivity.

*Feature*  
- Detection sensitivity of phosphorylated molecule is improved.

**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 from surfactants or reducing agents, phosphorylated protein can be obtained in a condition similar to in vivo one.

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

[Kit Contents]
- Phos-tag™ MS-101L ................. 5 mg  
- Phos-tag™ MS-101H  ................. 5 mg  
- Phos-tag™ MS-101N .............10 mg

[Application]
- Purification of phosphorylated proteins in A431 lysate  
  M : Molecular Marker  
  Lane 1 : Non absorbed fraction  
  Lane 2 : Absorbed fraction  
  Lane 3 : Column rinsing fraction  
  (Left) Fluorescence staining  
  (Right) Western blotting with anti-phosphorylated Tyr.
### Reagents for Phos-tag™ SDS-PAGE gel preparation

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No.</th>
<th>Use Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 w/v% Acrylamide solution, 29:1</td>
<td>500 mL</td>
<td>014-21705</td>
<td>Ready-to-use “Solution A”. 30%T, 3.3%C</td>
</tr>
<tr>
<td>Agarose H (High-strength type)</td>
<td>1 g</td>
<td>315-01203</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>10 g</td>
<td>319-01201</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 g</td>
<td>317-01202</td>
<td></td>
</tr>
<tr>
<td>10% SDS Solution</td>
<td>100 mL</td>
<td>311-90271</td>
<td>Ready-to-Use “Solution D”</td>
</tr>
<tr>
<td></td>
<td>500 mL</td>
<td>313-90275</td>
<td></td>
</tr>
<tr>
<td>Manganese(II) Chloride Tetrahydrate, 99.0+ % (Titration)</td>
<td>25 g</td>
<td>134-15302</td>
<td>for Molecular Biology</td>
</tr>
<tr>
<td></td>
<td>100 g</td>
<td>136-15301</td>
<td>Please use for preparation of “Solution F”</td>
</tr>
<tr>
<td>Zinc Chloride</td>
<td>25 g</td>
<td>268-01902</td>
<td>for Molecular Biology</td>
</tr>
<tr>
<td>Separating Gel Buffer Solution (x4)</td>
<td>250 mL</td>
<td>192-11041</td>
<td>Ready-to-Use mixed solution of “Sol. B” and “Sol. D” for preparation of Resolving Gel. Contains SDS.</td>
</tr>
<tr>
<td>Stacking Gel Buffer Solution (x4)</td>
<td>250 mL</td>
<td>199-11051</td>
<td>Ready-to-Use mixed solution of “Sol.C” and “Sol. D” for preparation of Stacking Gel. Contains SDS.</td>
</tr>
<tr>
<td>N,N,N',N'-Tetramethylethylenediamine (TEMED)</td>
<td>25 mL</td>
<td>205-06313</td>
<td>for Electrophoresis</td>
</tr>
<tr>
<td>10 w/v% Ammonium Peroxodisulfate Solution</td>
<td>25 mL</td>
<td>019-15922</td>
<td>Ready-to-Use “Solution G”</td>
</tr>
</tbody>
</table>

### Premixed Buffers

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No.</th>
<th>Use Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running Buffer Solution (x10)</td>
<td>1 L</td>
<td>184-01291</td>
<td>Ready-to-Use concentrated “Solution H”</td>
</tr>
<tr>
<td>SDS-PAGE 10x Running Buffer</td>
<td>1 L</td>
<td>312-90321</td>
<td>Ready-to-Use concentrated “Solution H”</td>
</tr>
<tr>
<td></td>
<td>5 L</td>
<td>318-90323</td>
<td></td>
</tr>
<tr>
<td>SDS-PAGE Buffer, pH 8.5</td>
<td>5 L</td>
<td>192-16801</td>
<td>Ready-to-Use “Solution H”, 1 x buffer.</td>
</tr>
<tr>
<td>Tricine Running Buffer Solution (x10)</td>
<td>1 L</td>
<td>200-17071</td>
<td>Composition: 0.5 M Tris / 0.5 M Tricine / 1% SDS</td>
</tr>
<tr>
<td>Sample Buffer Solution (2ME+) (x4)</td>
<td>25 mL</td>
<td>191-13272</td>
<td>Sample buffer for Laemmli SDS-PAGE containing 2-mercaptoethanol</td>
</tr>
<tr>
<td>Sample Buffer Solution (2ME+) (x2)</td>
<td>25 mL</td>
<td>196-11022</td>
<td></td>
</tr>
<tr>
<td>Sample Buffer Solution with 3-Mercapto-1,2-propanediol (x2)</td>
<td>25 mL</td>
<td>199-16132</td>
<td>Laemmli Sample Buffer containing 3-mercapto-1,2-propanediol (non-hazardous chemical) as substitute for 2-ME</td>
</tr>
<tr>
<td>Sample Buffer Solution with 3-Mercapto-1,2-propanediol (x2)</td>
<td>25 mL</td>
<td>196-16142</td>
<td></td>
</tr>
</tbody>
</table>
## Reagents for Staining

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No.</th>
<th>Use Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quick CBB Plus</td>
<td>250 mL</td>
<td>174-00553</td>
<td>Ready-to-Use \textit{Sol. K}. Fixing and destaining procedure are not required. No organic solvents are necessary. Protein bands are stained in 10 minutes.</td>
</tr>
<tr>
<td></td>
<td>1 L</td>
<td>178-00551</td>
<td></td>
</tr>
<tr>
<td>Quick-CBB</td>
<td>2 L</td>
<td>299-50101</td>
<td>By mixing staining solution A and B, ready-to-Use \textit{Sol. K}.</td>
</tr>
<tr>
<td>- Staining solution A: 1L × 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Staining solution B: 1L × 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver Stain MS Kit</td>
<td>20 tests</td>
<td>299-58901</td>
<td>Proteins are rarely modified chemically due to omitting treatment of glutaraldehyde and is detected at sub-nanogram level on the electrophoretic gel.</td>
</tr>
<tr>
<td>Silver Stain Kit Wako</td>
<td>for 10 gels</td>
<td>299-13841</td>
<td>50–100 times more sensitive than CBB method.</td>
</tr>
<tr>
<td>Silver Stain II Kit Wako</td>
<td>for 10 gels</td>
<td>291-50301</td>
<td>This kit contains Stopper, which can be adjusted the staining strength.</td>
</tr>
<tr>
<td>Negative Gel Stain MS Kit</td>
<td>20 tests</td>
<td>293-57701</td>
<td>Applicable for mass analysis and western blot</td>
</tr>
</tbody>
</table>

## Protein Size Marker

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No.</th>
<th>Use Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIDE-VIEW™ Prestained Protein Size Marker III</td>
<td>500 µL</td>
<td>230-02461</td>
<td>A recommendable prestained marker used with Phos-tag™ SDS-PAGE because obtained bands are less to distort.</td>
</tr>
</tbody>
</table>

## Enzyme for Dephosphorylation

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No.</th>
<th>Use Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase (for Biochemistry)</td>
<td>50 U</td>
<td>018-10693</td>
<td>Applicable for dephosphorylation of proteins</td>
</tr>
<tr>
<td></td>
<td>100 U</td>
<td>012-10691</td>
<td></td>
</tr>
</tbody>
</table>

## Electrophoresis Apparatus • Precast Gels

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Pkg. Size</th>
<th>Wako Cat. No.</th>
<th>Use Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasySeparator</td>
<td>1 set</td>
<td>058-07681</td>
<td>An electrophoresis tank for SuperSep precast polyacrylamide gels.*</td>
</tr>
<tr>
<td>SuperSep Ace, 12.5%, 13 well</td>
<td>10 gels</td>
<td>199-14971</td>
<td>Prior to use of SuperSep Phos-tag™ PAGE, please use these as sample confirmation. Expire in 9 months after the manufacture</td>
</tr>
<tr>
<td>SuperSep Ace, 12.5%, 17 well</td>
<td>10 gels</td>
<td>196-14981</td>
<td></td>
</tr>
<tr>
<td>SuperSep Ace, 15%, 13 well</td>
<td>10 gels</td>
<td>193-14991</td>
<td></td>
</tr>
<tr>
<td>SuperSep Ace, 15%, 17 well</td>
<td>10 gels</td>
<td>190-15001</td>
<td></td>
</tr>
</tbody>
</table>

*: Invitrogen’s electrophoresis tank is also applicable to SuperSep by using an adjuster. Please contact us.
Reagents for Western Blotting

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

* : Not available for sales in the US and Europe.
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: www.e-reagent.com

This brochure may contain products that cannot be exported to your country due to regulations.

Bulk quote requests for some products are welcomed. Please contact us.