FUJIFILM

Wako

Code No. 198-17981
195-17991

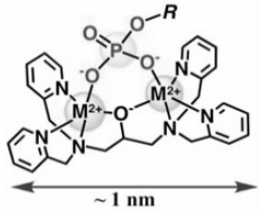
SuperSep™ Phos-tag™ (50 μmol/L), 83×100×3.9mm

SuperSep™ Phos-tag™ are precast polyacrylamide gels containing Phos-tag™ with zinc ion (Zn^{2+}). Phos-tag™ is a functional molecule that binds specifically to the phosphate group[1), 2), 3)]. This product can trap phosphorylated proteins during SDS-PAGE, and it allows detection of phosphorylated and non-phosphorylated proteins as different bands. The gels have a neutral pH to enhance stability.
This product is designed to be used with Mini-PROTEAN® Tetra Cell (#6580001, Bio-Rad Laboratories, Inc.).
Mini-PROTEAN® is a registered trademark of Bio-Rad Laboratories, Inc.

■ Function of Phos-tag™

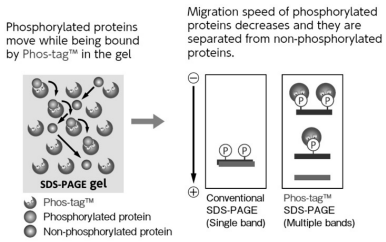
◇ Selectivity of binding of a phosphate ion is much higher than that of other anions.
◇ Stable complex is formed under physiological conditions (pH 5 to 8).

Basic structure of Phos-tag™



M^{2+} : manganese or zinc ion

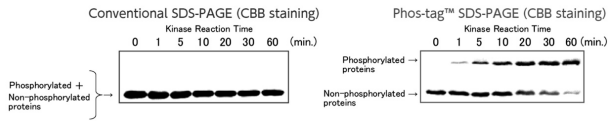
■ Principle of Phos-tag™ SDS-PAGE



■ Example of Phos-tag™ SDS-PAGE

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 (Abltide) and separated with conventional SDS-PAGE and Phos-tag™ SDS-PAGE, respectively.



< Note >

- Handle with care. The glass plates are fragile.
- This product is not suitable for measurement of protein molecular weight.
- The gel becomes clouded when being incubated with any buffer including methanol such as acetate-methanol solution for fixing proteins and transfer buffer. It does not affect the quality of the product.
- Handle the gel carefully not to tear it.
- It is not recommended to apply ordinary prestained protein ladders to polyacrylamide gels containing Phos-tag™ such as this product because the ladder patterns is disturbed. A prestained ladder recommended is WIDE-VIEW™ Prestained Protein Size Marker Ⅲ (Code No. 230-02461), which is designed to reduce disturbance of a ladder pattern in Phos-tag SDS-PAGE.

【Format】

Cassette size	83×100×3.9 (mm)
Max well volume	17 wells : 25 μL/well

【Package】
5 gels

【Storage】
Keep at 2 ～ 10℃. Do not freeze.

〔Additional materials required〕

- Running Buffer (×10) (Code No. 184-01291)
  ▶ 0.25 mol/L Tris, 1.92 mol/L Glycine, 1% SDS.
- Sample Buffer (2ME+) (×4) (Code No. 191-13272)
  ▶ 0.25 mol/L Tris-HCl, pH 6.8, 40 w/v% Glycerol, 8% SDS, 20 v/v% 2-Mercaptoethanol, 0.02 w/v% BPB.
- Electrophoresis apparatus - Mini-PROTEAN® Tetra Cell (#16580001, Bio-Rad Laboratories, Inc.)
- Disodium Dihydrogen Ethylenediamine Tetraacetate (EDTA)
- Transfer buffer (×10) : 10×Tris-Glycine Buffer (Code No. 201-18601)
  ▶ 0.25 mol/L Tris, 1.92 mol/L Glycine.
- Methanol (Code No. 131-01826)
- Blotting papers
- PVDF or nitrocellulose membrane (Code No. 033-22453, 032-22663)

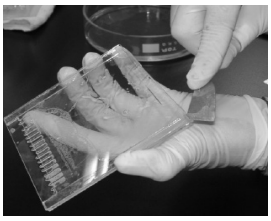
〔Procedure〕

1. Sample preparation for Phos-tag SDS-PAGE

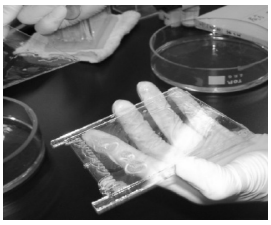
1) Remove contaminant in protein samples by TCA precipitation, dialysis or desalting*.
   * Phos-tag SDS-PAGE is vulnerable to contaminant in protein samples, especially chelating reagent, vanadic acid, inorganic salts, surfactants. Cleaning them up is strongly recommended before Phos-tag SDS-PAGE.
2) Mix sample with Sample Buffer Solution (2ME+) (×4) and heat for 5 min at 95℃. Cool it to room temperature.

2. Phos-tag SDS-PAGE

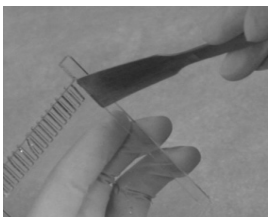
1) Remove a gel cassette from a pouch and set it into the electrophoresis apparatus.
2) Fill the reservoir with 1× running buffer.
3) Carefully remove the comb from the gel.
4) Load the samples into lanes. Blank lanes may cause distortion of bands. Load the same amount of 1× sample buffer into blank lanes.
5) Start electrophoresis, according to the instruction of the electrophoresis apparatus.
6) Remove the gel cassette from the electrophoresis apparatus and rinse the gel cassette with water.
7) Insert a gel knife into a bottom slit of gel cassette. Rever up the one side gel plate.



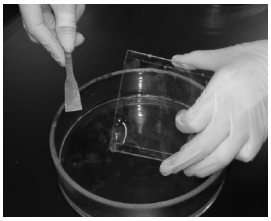
8) Carefully remove the one side gel plate, while the gel remains on the other plate.



9) Insert a gel knife into the slit between the gel and gel plate, and cut pressingly the gel. In the same way, cut pressingly the gel at left and right sides.



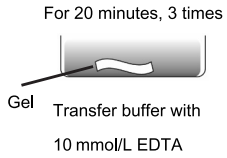
10) Turn it upside down over a dish and detach the gel with the help of the knife.



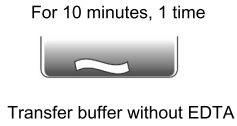
3. Pre-treatment for Transfer

An additional procedure, elimination of the zinc ion (Zn^{2+}) from the gel using EDTA, is necessary before transfer. This procedure increases transfer efficiency of proteins from a gel to a membrane.

1) Prepare 1× transfer buffer with 10 mmol/L EDTA and without EDTA.
   ▶ 1× transfer buffer with 10 mmol/L EDTA
   25 mmol/L Tris, 0.192 mol/L Glycine, 10 v/v% methanol and 10 mmol/L EDTA.
   ▶ 1× transfer buffer without 10 mmol/L EDTA
   25 mmol/L Tris, 0.192 mol/L Glycine and 10 v/v% methanol.
2) Soak the gel in 1× transfer buffer with 10 mmol/L EDTA for a minimum of 20 minutes with gentle agitation. Repeat it 3 times with buffer exchanges.



3) Soak the gel in 1× transfer buffer without 10 mmol/L EDTA for 10 minutes with gentle agitation.



4) Transfer the proteins from the gel to a membrane*.
   * A Wet-tank method is strongly recommended for effective protein transfer.

【References】

1) Kinoshita, E., Kinoshita-Kikuta, E., Takiyama, K. and Koike, T. : *Mol. Cell. Proteomics*, **5**, 749 (2006).
2) Yamada, S., Nakamura, H., Kinoshita, E., Kinoshita-Kikuta, E., Koike, T. and Shiro, Y. : *Anal. Biochem.*, **360**, 160 (2007).
3) Kinoshita-Kikuta, E., Aoki, Y., Kinoshita, E. and Koike, T. : *Mol. Cell.* Proteomics, **6**, 356 (2007).

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