Phos-tag™ Yellow

- Phosphoprotein Gel Staining -

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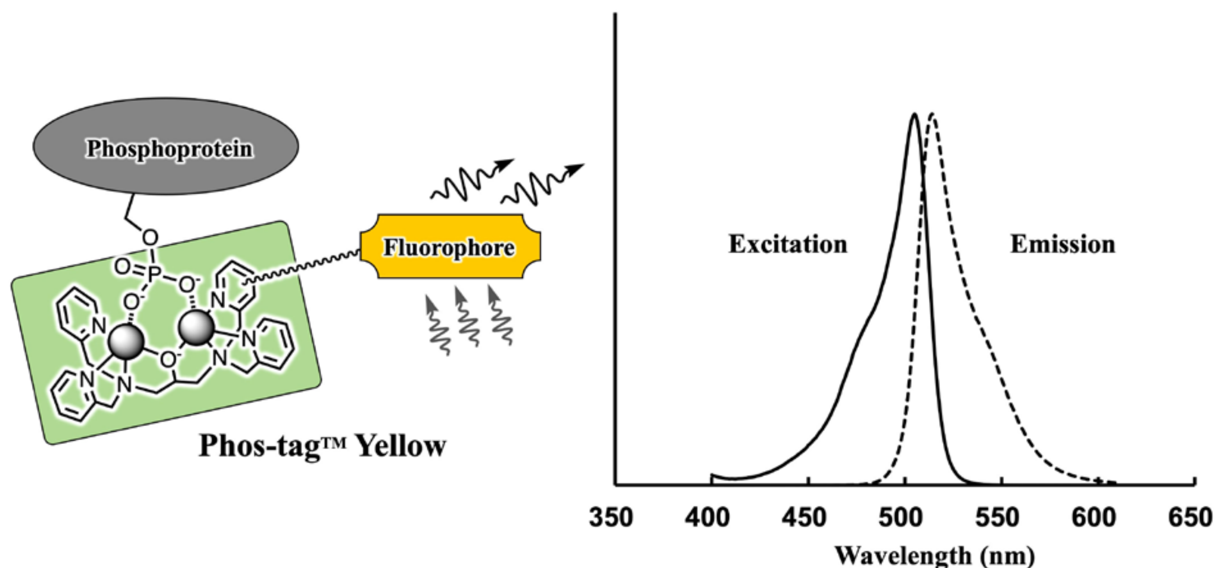
Phos-tag™ Yellow Staining of Phosphoproteins

1. Introduction

Phosphorylation is a fundamental covalent post-translational modification that regulates the function, localization, and binding specificity of target proteins. Methods for determining the phosphorylation status of proteins are thus very important with respect to the evaluation of diverse biological and pathological processes. In 2002, Prof. Koike's group (Hiroshima University) reported a selective phosphate-binding tag molecule, Phos-tag™. Since then, various methods for phosphoproteome research have been developed using Phos-tag™ derivatives. Here, we demonstrate an application of Phos-tag™ for a fluorometric detection of phosphoproteins in a polyacrylamide gel by using Phos-tag™ Yellow.

2. Description of Phos-tag™ Yellow and Phos-tag™ Common Solution

A fluorescent Phos-tag™, Phos-tag™ Yellow, provides a preferential detection of phosphorylated proteins in a SDS-PAGE gel. The product is supplied as an orange solid placed in a plastic microtube. Phos-tag™ Common Solution (5x: 5-fold concentrated, neutral pH) is used for the preparation of "Equilibrating & Washing Solution" and "Staining Solution". Below 4˚C, the both products are stable for at least 6 months.



3. Warning and Limitations

Phos-tag™ Yellow and Phos-tag™ Common Solution are not for use in human diagnostic and the therapeutic procedures. Do not use internally or externally in human or animals. Those are used only for research. Care should be taken to avoid contact with skin or eyes. We recommend the use of plastic gloves. In the case of contact with skin or eyes wash immediately with water.

4. Advantages of Phos-tag™ Yellow Staining

The gel staining involves a simple procedure using two solutions in the basic protocol.
The total operation time is very short, *ca.* 2 h at room temperature.
Radioactive phosphorus, chemical labels, and antibody are not required.
Phos-tag™ Yellow binds preferentially to phosphorylated amino acid residues.
Downstream procedures such as CBB staining and MS analysis are applicable.
Phosphoproteins separated by Phos-tag™ SDS-PAGE can be also visualized by Phos-tag™ Yellow.
A highly-negative (strong acidic) protein such as monophosphorylated pepsin is more selectively detected by using Phos-tag™ Yellow in comparison with Phos-tag™ Magenta and Phos-tag™ Cyan.

5. Solutions for Phos-tag™ Yellow Gel Staining

∗**Equilibrating & Washing Solution**: ~1 L

# Phos-tag™ Common Solution (5x)	100 mL
# pure water	400 mL
# methanol	500 mL

∗**Staining Solution**: ~1 L

# Phos-tag™ Yellow	Entire content in 1 microtube
# Phos-tag™ Common Solution (5x)	100 mL
# pure water	400 mL
# methanol	500 mL

Note: Phos-tag™ Yellow should be completely dissolved with 1 mL methanol in the microtube and then the solution is diluted with 499 mL of methanol, 100 mL of Phos-tag™ Common Solution (5x), and 400 mL of pure water.

Caution: Phos-tag™ Yellow gradually adsorbs on glass and aromatic polymer products such as poly(styrene) and poly(ethylene phthalate) bottles, so the staining solution should be contained in **a polypropylene or polyethylene bottle**. The bottle should be stored in the dark at 4°C before use. The adsorption decreases the concentration of Phos-tag™ Yellow resulting in reduce of fluorescence intensity of phosphoproteins. After preparation of Phos-tag™ Yellow staining solution, we recommend that it is used within 6 months.

6. Basic Protocol for Phos-tag™ Yellow Gel Staining (Figure 1)

Step 1) After electrophoresis of the target phosphoprotein on a polyacrylamide mini-gel (90 x 80 x 1 mm^3), the gel is placed in a staining tray, completely covered with *Equilibrating & Washing Solution* (50 mL) and incubated at room temperature with gentle shaking for 15 min. After the solution is decanted, the same equilibration is conducted again for 15 min.

Step 2) The equilibrating solution is decanted, and the gel is completely covered with *Staining Solution* (50 mL) and incubated at room temperature with gentle shaking for 60 min.

Step 3) The staining solution is decanted, and as much as possible of the residual solution in the staining tray is absorbed on a paper towel. The gel is washed in the tray by gentle shaking with *Equilibrating & Washing Solution* (50 mL) at room temperature for 30 min.

Step 4) Set the gel on the sample stage of a fluorescence imaging apparatus (excitation at 473 nm with a 510-nm longpass emission filter), and then visualize the phosphoprotein. If the imaging of total proteins is needed, CBB staining can be conducted subsequently without pretreatment of the polyacrylamide gel (see Figures 2 & 3).

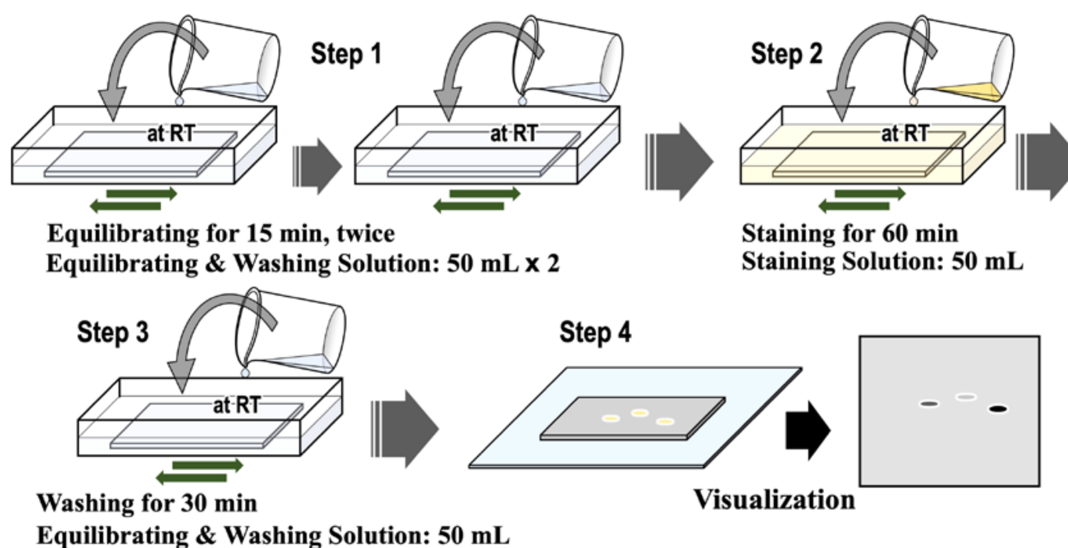


Figure 1. Step-by-step procedure for Phos-tag™ Yellow staining.

Note: Phos-tag^{TM} Yellow gradually adsorbs on glass and aromatic polymer products such as poly(styrene) and poly(ethylene phthalate) staining tray, so the staining is conducted using **a polyethylene or polypropylene tray**. If the Phos-tag^{TM} Yellow adsorbed on a labware, it can be removed by treatment with an aqueous solution of NaClO and a sufficient amount of water.

In all the steps, the staining tray is covered with aluminum foil (or a black box) to protect from light.

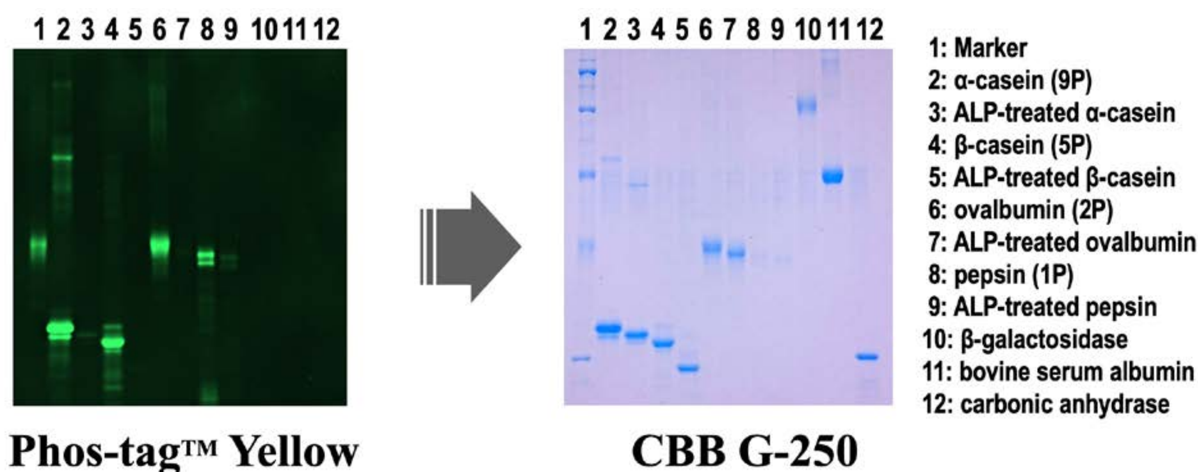


Figure 2. Selectivity of the Phos-tag^{TM} Yellow stain: A 10% (w/v) polyacrylamide gel containing four sets of intact and ALP-treated phosphoproteins (1 μg each of α-casein, β-casein, ovalbumin, and pepsin) was stained with Phos-tag^{TM} Yellow (left) and subsequently with CBB G-250 gel satin (right). The phosphoproteins were selectively visualized by using a fluorescence imaging scanner, FLA-5000 (Fujifilm Life Science) at 473-nm excitation with a 510-nm longpass emission filter.

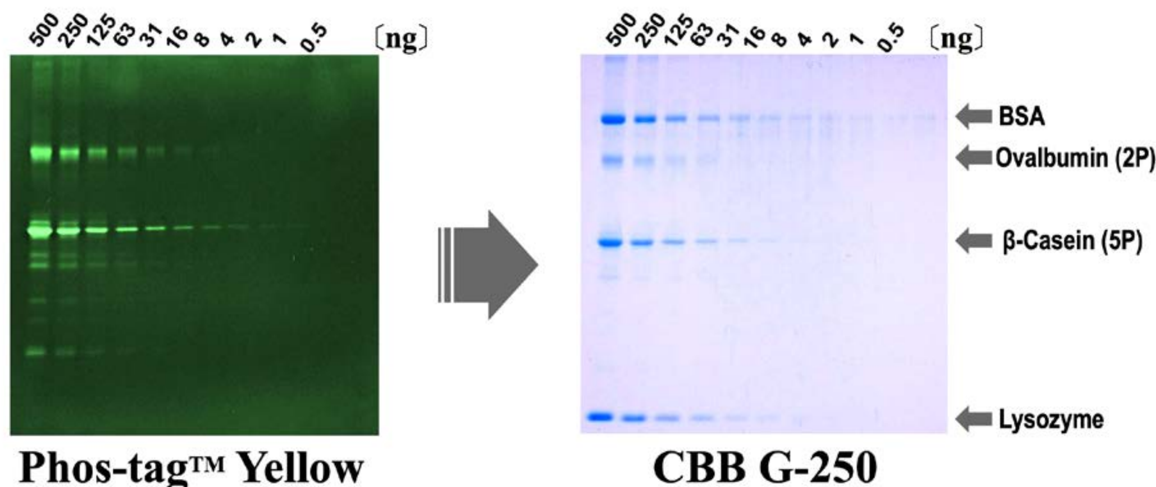


Figure 3. Selectivity of the Phos-tag^{TM} Yellow stain: A 12.5% (w/v) polyacrylamide gel containing phosphorylated and nonphosphorylated proteins was stained with Phos-tag^{TM} Yellow (left) and subsequently with CBB G-250 gel satin (right). The sample contained equivalent amounts of two pSer-phosphoproteins (ovalbumin and β-casein) and two nonphosphorylated proteins (bovine serum albumin [BSA] and lysozyme). The total amounts of protein are shown above each lane. The phosphoproteins were selectively visualized by using a fluorescence imaging scanner, FLA-5000 (Fujifilm Life Science) at 473-nm excitation with a 510-nm longpass emission filter.

Note: We found so far that some highly-positive (basic) phosphoproteins (*e.g.*, phosphorylated Histone H1 and Myelin basic protein) are hardly detectable by using Phos-tag^{TM} Yellow. The reason in detail is not clear, but an ion-pairing between $-PO_3^{2-}$ and a positive-charged residue (Lys or Arg) and/or an electrostatic repulsion between the 3+-charged Phos-tag^{TM} moiety and the highly-positive phosphoproteins might inhibit the phosphate-binding of Phos-tag^{TM} Yellow.

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