**Phos-tag Magenta - Phosphoprotein Gel Staining**

<https://labchem-wako.fujifilm.com/us/product_data/docs/386-15221.pdf>

A fluorescent PhosTag, PhosTag Magenta, provides a preferential detection of phosphorylated proteins in a SDS-PAGE gel. The product is supplied as an red solid placed in a plastic microtube. PhosTag 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 month

**Solutions for PhosTag** **Magenta Gel Staining**

* Equilibrating & Washing Solution: ~1 L

PhosTag Common Solution (5x) 100 mL

Di water 400 mL

Methanol 500 mL

* Staining Solution: ~1 L

PhosTag Magenta Entire content in 1 microtube

PhosTag Common Solution (5x) 100 mL

Di water 400 mL

Methanol 500 mL

Note: PhosTag Magenta 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 PhosTag Common Solution (5x), and 400 mL of purified water.

**Stain Procedure (Figure 1):**

1. After electrophoresis of the target phosphoprotein on a polyacrylamide mini-gel (90 x 80 x 1 mm3), 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.
2. The equilibrating solution is decanted, and the gel is completely covered with Staining Solution (50mL) and incubated at room temperature with gentle shaking for 60 min.
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.
4. Set the gel on the sample stage of a fluorescence imaging apparatus (excitation at 532 nm with a 575-nm long-pass 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 gel (see Figure 2).

Note: PhosTag Magenta 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 PhosTag Magenta 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.

A diagram of a step-by-step method

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A close-up of a test

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