

GelCode[®] Phosphoprotein Staining Kit

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Product Description

Number

24550

Description

GelCode[®] Phosphoprotein Staining Kit

Kit contains sufficient materials to stain 10 (8 x 8 cm) SDS-PAGE gels.

Kit includes the GelCode[®] Phosphoprotein Stain Reagent Set (Product No. 24551) and Phosphoprotein Control Set (Product No. 24552).

Kit Components:

24551

GelCode[®] Phosphoprotein Stain Reagent Set

Reagent 1: Sulfosalicylic Acid Solution, 1,000 ml

Reagent 2: Sulfosalicylic Acid + CaCl₂ Solution, 250 ml

Reagent 3: 0.5 N NaOH, 250 ml

Reagent 4: Ammonium Molybdate Solution, 500 ml

Reagent 5: Ammonium Molybdate + HNO₃ Solution, 250 ml

Reagent 6: Methyl Green Solution, 250 ml

Reagent 7: 7% Acetic Acid, 500 ml

24552

Phosphoprotein Control Set

Positive Control (Phosvitin), 1 mg (~ 40 kD in gel)

Negative Control (Soybean Trypsin Inhibitor), 1 mg (~ 20 kD in gel)

Introduction

Protein phosphorylation and dephosphorylation play significant regulatory roles in a variety of cellular processes such as normal and abnormal cell growth, cell death, and secretion. As a result, it has become increasingly important to detect changes in the phosphorylation status of proteins.

GelCode[®] Phosphoprotein Staining Kit is designed for specific staining of phosphorylated proteins directly on SDS-PAGE gels. The method depends on the hydrolysis of the phosphoprotein phosphoester linkage using 0.5 N NaOH in the presence of calcium ions. The reagents in this kit hydrolyze the phosphoester linkage of phosphoserine and phosphothreonine. Phosphotyrosine is not hydrolyzed. For this reason, phosphotyrosine cannot be detected with this kit. The gel containing the newly formed insoluble calcium phosphate is then treated with ammonium molybdate in dilute nitric acid. The resultant insoluble nitrophospho-molybdate complex is finally stained with the basic dye, Methyl Green Solution.

The limit of detection with this staining kit depends on several factors, including the molar amount of phosphate loaded onto the gel and the accessibility of these groups within the electrophoresed sample to hydrolysis during the staining procedure.

For several proteins, the calculated lower molar limit of phosphate detection varied more than five-fold (Table 1). These results indicate that lower limits of detection must be determined empirically for each phosphoprotein.

Preparation of Controls:

1. Dissolve contents of each phosphoprotein control vial in 1 ml Tris Buffered Saline, 25 mM Tris, 180 mM NaCl, pH 7.2 (Pierce Product No. 28376, 27379) to yield 1 mg/ml (1 $\mu\text{g}/\mu\text{l}$) stock solutions.
2. Prepare a 10-fold dilution to yield a 0.1 $\mu\text{g}/\mu\text{l}$ working solution. Further dilutions can also be used.
3. Load 2-10 μl of controls after mixing and heating with an appropriate volume of gel sample loading buffer.

Note: We recommend loading several concentrations (e.g., 10, 1 and 0.1 μg) of positive control to ensure appropriate sensitivity of kit and detection range for one's sample.

Procedure:

Note: Avoid touching the gel or allowing it to contact dirty labware throughout the procedure as these will result in high background. The entire procedure can be carried out in one clean staining tray; simply pour off one reagent (without touching the gel) and add the next. Use sufficient volumes of reagents to ensure that the gel is kept wetted across its entire surface for the duration of each incubation.

1. Pre-heat an incubation oven to 65°C in preparation for step 6. Substituting a water bath for this step produces poor results and is not recommended.
2. After electrophoresis, transfer the gel to a clean gel tray, add 50 ml of deionized water and wash the gel by gentle agitation for 10 minutes.
3. Pour off the water; add 25 ml of Reagent 1 and gently agitate the gel for 15 minutes.
4. Pour off Reagent 1; add 25 ml of Reagent 2 and gently agitate the gel for 30 minutes.
5. Pour off Reagent 2; quickly rinse the gel with deionized water to remove any surface CaCl_2 .
6. Pour off the rinse water; add 25 ml of Reagent 3, cover the tray with a lid, and place it in an oven 65°C oven for 20 minutes. It is not necessary to agitate the gel during this step.

Note: Because the gel will swell to approximately 110% of its original size in this step, be sure that the tray is large enough to accommodate this increase comfortably. The tray containing the gel must be covered with a lid to prevent evaporation and uneven wetting to the gel, which can result in high background. Formation of a white precipitate is normal during this step.

7. Pour off Reagent 3; add 25 ml of Reagent 4 and gently agitate the gel for 10 minutes. Repeat this step once.
8. Pour off Reagent 4; add 25 ml of Reagent 5 and gently agitate the gel for 20 minutes.
9. Pour off Reagent 5; add 25 ml of Reagent 6 and gently agitate the gel for 20 minutes.
10. Pour off Reagent 6; destain the gel by adding 25 ml of Reagent 1 and gently agitating the gel for 15 minutes. Repeat this step once. At this stage, a green band of phosphoprotein can be visualized against a faint green background.
11. Completely destain the gel in 25 ml of Reagent 7 and incubate overnight with gentle agitation. Change the solution once after it becomes green in color.

Note: The gel can be dried after first incubating it in gel drying solution (5% glycerol and 10% ethanol in water) for at least 5 hours. Alternatively, the gel may be stained for total proteins using GelCode® Blue Stain Reagent (Product No. 24590 or 24592) after first washing it 3 x 10 minutes in 100 ml of deionized water.

Table 1: Lower limits of detection for several phosphoproteins using the GelCode® Phosphoprotein Stain Reagent Set.

Protein	Phosvitin	β -casein	Ovalbumin	Histone Type III-S
Lower limit of detection (total phosphoprotein)	0.080 μ g	0.160 μ g	10.0 μ g	10.0 μ g
% phosphate by weight	10%	1%	0.12%	0.02%
Molar ratio (phosphate groups to protein)	100:1	4.39:1	1.73:1	0.26:1
Lower limit of detection (weight of phosphate)	8 ng	1.6 ng	12 ng	2 ng
Lower limit of detection (moles of phosphate)	101 pmoles	20 pmoles	151 pmoles	25 pmoles

Staining for Total Proteins with GelCode® Blue Stain Reagent (Product No. 24590 or 24592)

After staining the phosphoproteins, it is possible to stain total proteins using the GelCode® Blue Stain Reagent.

Method

1. Wash the phosphoprotein-stained gel with 100 ml of deionized water 3 times for 10 minutes each.
2. Place the gel in 50 ml of GelCode® Blue Stain Reagent and gently agitate for 1 hour.
3. Wash the gel with deionized water.

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