Phos binding reagent (Phosbind) acrylamide

Introduction

Phosbind Acrylamide is an innovative phosphate-binding reagent. It appears as a yellow, viscous oil and belongs to a class of acrylamide derivatives functionalized with the Phosbind ligand, which are also bicyclic metal complexes. It is specifically designed for the electrophoretic separation and detection of phosphorylated and non-phosphorylated proteins. In the presence of divalent metal ions such as Mn^{2+} or Zn^{2+}, this reagent specifically recognizes and binds to phosphate groups, with the optimal reaction condition at neutral physiological pH 6–8.

The mechanism of Phosbind Acrylamide is based on its specific interaction with phosphate groups. After incorporating this reagent together with divalent metal ions (e.g., Mn^{2+} or Zn^{2+}) into a conventional SDS-PAGE separating gel, the Phosbind Acrylamide-metal ion complex immobilized in the gel specifically binds to the phosphate groups on proteins. This slows down the electrophoretic mobility of phosphorylated proteins, while the migration of non-phosphorylated proteins remains unaffected, thereby achieving separation between the two. The higher the phosphorylation level of a protein, the slower its migration rate. The typical molar ratio of Phosbind Acrylamide to metal ions is 1:2, although exceptions may exist in practical applications.

This product has a wide range of applications. It can be used for the qualitative and quantitative detection of phosphorylation levels in in vitro kinase and phosphatase reaction systems, the analysis of endogenous protein phosphorylation, the study of plant protein phosphorylation, the identification of protein phosphorylation states, and phosphoproteomics research. It can also achieve fine separation of phosphorylated isoforms in two-dimensional electrophoresis. Furthermore, it is suitable for drug screening and target validation, cell signaling pathway analysis (e.g., GPCR, Wnt, MAPK), as well as basic research fields such as neuroscience, metabolic regulation, apoptosis, and DNA damage repair.

This product is suitable for detecting proteins within the molecular weight range of 5–350 kDa. It has no species restrictions (human, animal, plant, microorganism, etc.) or sample type restrictions (purified proteins, cell lysates, tissue homogenates, in vitro kinase reaction systems, etc.). It is compatible with various downstream analysis methods, including CBB staining, Western Blotting (WB), two-dimensional electrophoresis, and mass spectrometry (MS). Compared to traditional phosphorylation detection methods, this technology can directly visualize phosphorylation-dependent electrophoretic mobility shifts without the need for specific phosphorylation antibodies. For better experimental results, it is recommended to use this product with supporting reagents, including Prestained Protein Marker (F4005), Protease Inhibitor Cocktail (K1007), Phosphatase Inhibitor Cocktail (K1015), and Tris-Gly Fixed-Concentration Gel Preparation Kits (K4131-K4135, 6%/8%/10%/12%/15%).

This product consists of Phosbind Acrylamide and a separately packaged tube of $MnCl_2$. Taking a common Mini PAGE gel (8.3 cm × 7.3 cm, 1 mm thickness) as an example, based on a separating gel volume of 5 mL and a final Phosbind Acrylamide concentration of 50 μM, the 5 mg specification can prepare approximately 33 gels. The number of preparable gels varies with the concentration of Phosbind Acrylamide used; the higher the concentration, the fewer gels can be prepared.

Components and Storage

Components	2 mg	5 mg	10 mg	Storage
Phos binding reagent (Phosbind) acrylamide	2 mg	5 mg	10 mg	4°C away from light
$MnCl_2$	100 mg	100 mg	100 mg	4°C
Shipping: Blue ice	**Shelf life: 12 months**			

Protocol

1. Phosbind Acrylamide System Preparation

   a. Preparation of Phosbind Acrylamide Stock Solution (5.0 mmol/L, 3% (v/v) Methanol)

Component	Size 1	Size 2	Size 3
Phos binding reagent (Phosbind) acrylamide	2 mg	5 mg	10 mg
Methanol	20 μL	50 μL	100 μL
H_2O	640 μL	1.6 mL	3.2 mL

***Note: 1.** The Phos binding reagent (Phosbind) acrylamide should be completely dissolved in methanol first, then diluted with an appropriate amount of water to prepare the stock solution. 2. The prepared stock solution should be wrapped in aluminum foil and stored at 4°C protected from light. It can be stored stably for at least three months.

   b. Preparation of $MnCl_2$ Working Solution (10 mmol/L)

Component	Amount
$MnCl_2(H_2O)_4$ (MW: 198)	100 mg
H_2O	50 mL

***Note:** 1. Avoid using other anion salts (e.g., $Mn(NO_3)_2$ or $Mn(CH_3COO)_2$), because in alkaline aqueous solutions, these salts will first form a white $Mn(OH)_2$ precipitate, which then oxidizes to brown MnO(OH). This not only colors the gel but also renders the Mn^{2+} ineffective. 2. Store the prepared $MnCl_2$ working solution at 4°C.

2. Gel Preparation

   a. Preparation of Separating Gel: Refer to the following formula to prepare 10 mL of separating gel at different concentrations (single gel requires 5 mL, thus enough for 2 gels).

Component	25 μM （Phosbind Acrylamide）				50 μM （Phosbind Acrylamide）				100 μM （Phosbind Acrylamide）			
Gel Concentration (w/v)	12%	10%	8%	6%	12%	10%	8%	6%	12%	10%	8%	6%
Acr-Bis (30%, 29:1) (mL)	4	3.33	2.67	2	4	3.33	2.67	2	4	3.33	2.67	2
Tris-HCl (1.5 mol/L, pH 8.8) (mL)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Phosbind Acrylamide Stock Solution (mL)	0.05	0.05	0.05	0.05	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
$MnCl_2$ Working Solution (mL)	0.05	0.05	0.05	0.05	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
SDS (10% w/v) (mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
H2O (mL)	3.24	3.91	4.57	5.24	3.14	3.81	4.47	5.14	2.94	3.61	4.27	4.94
APS (10% w/v) (mL)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
TEMED (mL)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Total (mL)	10	10	10	10	10	10	10	10	10	10	10	10

Parameter Adjustments for Gel Preparation:

- Phosbind Acrylamide Concentration Optimization: For first-time use, it is recommended to start with a low concentration (e.g., 25 μM, which has broad applicability), then gradually test from low to high to determine the optimal concentration, such as 25 μM → 50 μM → 100 μM, or refer to the literature to select an appropriate concentration and make fine adjustments. Generally, for complex samples (e.g., cell lysates), the concentration should be between 5-25 μM. However, if the protein concentration is very low, the concentration can be increased, e.g., to 100 μM. For proteins with large molecular weights, a low concentration (e.g., 20–50 μM) is recommended, together with a low-concentration (agarose) gel.
- Optimization of Phosbind Acrylamide to Metal Ion Ratio: The ratios in the table are based on a typical molar ratio of Phosbind Acrylamide to Mn^{2+} of 1:2. However, exceptions may exist in practice (especially when the sample contains interfering components such as EDTA). Please adjust accordingly.
- Optimization of Separating Gel Concentration: Generally, use 10% gel for molecular weight < 20 kDa, 8% gel for 20-60 kDa, 6% gel for > 60 kDa, and 3-4% gel with 0.5% agarose for very large proteins (e.g., 200-350 kDa). Please adjust the specific concentration according to actual results.

b. Preparation of Stacking Gel (4.5% w/v):

Component	2 mL Volume	4 mL Volume (commonly used)	10 mL Volume
Acr-Bis (30%, 29:1) (mL)	0.3	0.6	1.5
Tris-HCl (0.5 mol/L, pH 6.8) (mL)	0.5	1.0	2.5
SDS (10% w/v) (μL)	20	40	100
H_2O (mL)	1.17 (exactly 1.168)	2.34 (exactly 2.336)	5.84
APS (10% w/v) (μL)	10	20	50
TEMED (μL)	2	4	10
Total (mL)	2	4	10

Parameter Adjustments for Gel Preparation:

- Stacking Gel Concentration Optimization: For high molecular weight proteins, prepare the stacking gel with 0.5% agarose, consistent with the separating gel.

***Note:** Our gel preparation kits can be used together: Tris-Gly Fixed-Concentration Gel Preparation Kits (K4131-K4135, 6%/8%/10%/12%/15%, 5 concentration products). For gel preparation kits from other brands, it is recommended to test before use.

3. Sample Processing

a. Mix the sample with protein loading buffer, heat at 95°C for 5 minutes, then cool to room temperature. 5X Protein Loading Buffer (Reducing) (K1164) is recommended.

***Note:** 1. For samples containing EDTA/inorganic salts/surfactants: TCA precipitation or dialysis desalting is recommended. 2. For samples with high viscosity (e.g., over-confluent cell lysates): thorough boiling or dilution is recommended. 3. For acidic samples (e.g., appearing yellow-orange after adding loading buffer): neutralize with Tris to neutrality until the color becomes blue-purple.

b. Loading: 1–5 μg for purified proteins, 10-30 μg for cell/tissue lysates (adjust according to expression level). For protein markers, it is recommended to use EDTA-free and non-phosphorylated markers. Prestained Protein Marker (Triple color, EDTA free, 10-250 kDa) (F4005) is recommended.

4. Electrophoresis (Choose One)

- First Choice (Constant Current, Recommended): Run the samples under constant current conditions (25-30 mA/gel), optionally with active heat dissipation (e.g., placed in a 4°C cold room or ice bath), until the bromophenol blue (BPB) reaches the bottom of the separating gel. Adjustable as needed.

- Second Choice (Constant Voltage): Choose this when there are special heat dissipation requirements or when the Phosbind Acrylamide concentration is very high. First, run the samples at 70 V constant voltage until the samples have completely entered the separating gel, then increase the voltage to 140 V and stop electrophoresis when the bromophenol blue indicator runs out of the bottom of the gel. Adjustable as needed. Active heat dissipation (e.g., placed in a 4°C cold room or ice bath) can also be optionally applied.

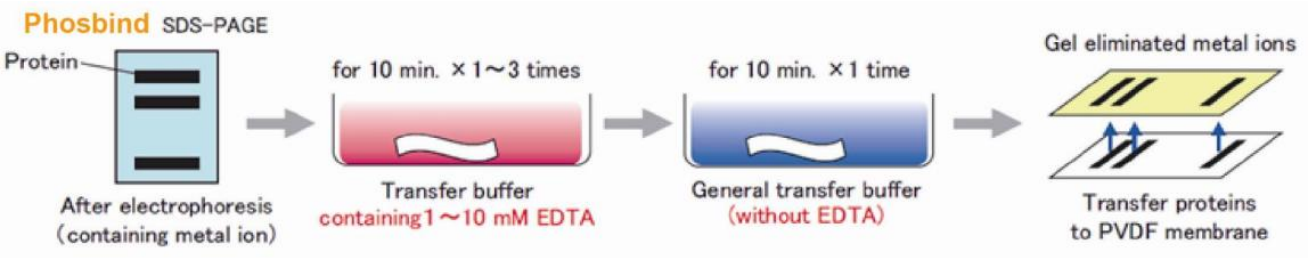
5. CBB Staining and Destaining

a. Staining: Remove the gel after electrophoresis, immerse it in CBB staining solution, and place on a shaker with gentle agitation for 2-10 minutes. Rapid Protein Stain (CBB Method, B8226) is recommended.

b. Destaining: Shake and rinse with water for 3-5 minutes for destaining. Alternatively, add sufficient water to completely cover the gel, then use a microwave oven at medium-high heat to accelerate destaining. Closely monitor and change the water multiple times until the background is clean and the bands are clear.

6. Immunoblotting

a. Pre-transfer Treatment: After electrophoresis, first immerse the gel in ordinary transfer buffer containing 1-10 mmol/L EDTA with gentle shaking for no less than 10 minutes. Repeat this step 1-3 times. Then replace with EDTA-free transfer buffer and gently shake for 10 minutes for washing.



b. Transfer: Wet transfer is recommended. Follow常规 immunoblotting experimental procedures for subsequent steps.

Note

1. For other specific details, please refer to the detailed protocol in another version.
2. This product is for research use only.

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