

Product Data Sheet

Product Name: Phosbind Biotin BTL-104

Cat. No.: GK10006

Components

Components	Formula	M.Wt	Solubility
Phosbind Biotin BTL-104	C ₄₀ H ₅₀ N ₁₀ O ₄ S	766.95	DMSO: >32.3 mg/mL
ZnCl ₂	ZnCl ₂	136.3	Water: >2 mg/mL

Features

Applications	● No radiation. ● No need for PVDF membrane sealing treatment. ● Phosbind The specific binding of is independent of the type and sequence of amino acids. ● Applicable for subsequent work such as immunoblotting and mass spectrometry analysis. ● Phosbind The BTL mother liquor can be stably stored for at least 6 months. ● The experimental process is similar to using HRP labeled antibodies.
Shipping	Blue ice transportation or according to your needs.
Storage	2-8 °C, stable for up to 12 months.
Usage	For Research Use Only! Not For Use in Humans.

Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1、 Solutions for Phosbind Biotin BTL-104 Method

Sol. A : Tris buffered saline	Tris buffered saline (10xTBS, 1 L, pH 7.5) Tris (0.10 mol/L) -----12.1 g NaCl (1.0 mol/L) -----58.4 g distilled water-----0.9 L 2 mol/L aqueous HCl for pH adjustment at 7.5-----a proper quantity distilled water for preparation of the 1 L solution-----a proper quantity
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Sol. B: Tween 20 solution	10% (v/v) Tween 20 solution (50 mL) Tween 20 -----5 mL distilled water -----45 mL
Sol. C: TBS-T	1xTBS-T (1 L) Sol. A -----100 mL Sol. B -----10 mL distilled water -----890 mL
Sol. D: Phosbind Biotin BTL-104 methanol solution	Phosbind Biotin BTL-104 methanol solutions Phosbind Biotin BTL-104 (MW: 767)-----10 mg Methanol-----0.13 mL 【Storage】 Stored in a dark place at 4°C.
Sol. E: Phosbind Biotin BTL-104 solution	Phosbind Biotin BTL-104 solution (5mmol/L) Sol. D-----0.13 mL Distilled water-----2.47mL 【Storage】 Stored in a dark place at 4°C.

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Sol. F: ZnCl ₂ aqueous solution	10 mmol/L ZnCl ₂ aqueous solution (50 mL) ZnCl ₂ (FW. 136.3)----- 68.15 mg Distilled water -----50 mL
Sol. G: Streptavidin-conjugated Horseradish Peroxidase solution	Streptavidin-Horseradish Peroxidase Conjugate

2、 Preparation of Phosbind Biotin-Streptavidin-conjugated HRP

1) After mixing of the following solutions, the obtained solution (Sol. H) is allowed to stand for 30 min at room temperature.

Sol. C (1xTBS-T) -----459uL

Sol. E (Phosbind Biotin BTL-104 solution) *----- 2 ~ 20 µL

Sol. F (10 mmol/L ZnCl₂)----- 2 ~ 20 µL

Sol. G (Streptavidin-conjugated Horseradish Peroxidase) ----- 1 µL

2) Sol. H is added in a centrifugal filter device cup (NMWL = 30,000, NanosepTM 30K). Seal with the attached cap.

3) Centrifuge (14,000 x g) for 20 min at room temperature to remove the excess Phosbind Biotin BTL-104.

4) The remaining solution (<10 µL) in the cup is diluted with 30 mL of Sol. C (1xTBS-T), which is Sol. Phosbind Biotin-SH (a solution of Zn²⁺-Phosbind Biotin-bound Streptavidin-conjugated HRP^{**}).

*: [Phosbind Biotin BTL-104] >> [Streptavidin-conjugated HRP]

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: Phosbind Biotin-bound Streptavidin-conjugated HRP in Sol. Phosbind Biotin-SH is stable for 30 days at 4 °C

3、 Probing with Phosbind Biotin-bound Streptavidin-conjugated HRP

1) A protein-blotted PVDF membrane is soaked with Sol. C (1xTBS-T) in a Tupperware. Use plastic gloves in this procedure. The membrane is gently rocked for at least 1 h. Confirm that the membrane does not stick to the Tupperware. Be careful not to dry the membrane.

2) The membrane is incubated with Sol. Phosbind Biotin-SH (ca. 1 mL/5 cm²) in a plastic bag. The bag is gently rocked for 30 min.

3) The membrane is taken out of the bag and washed twice with Sol. C (ca. 10 mL/5 cm²) in a Tupperware for 5 min each time at room temperature (the Tupperware is gently rocked). Confirm that the membrane does not stick to the Tupperware. Be careful not to dry the membrane.

4) The chemiluminescence is observed using an X-ray film or an image analyzer with an appropriate amount of a chemiluminescence reagent (e.g., Ultra High Sensitivity ECL Kit GK10008).

4、 Reprobing the Protein-blotted PVDF Membrane

Sol. K: Tris-HCl buffer	0.5 mol/L Tris-HCl buffer (1 L, pH 6.8) Tris ----- 60.6 g distilled water-----0.8 L 6 mol/L aqueous HCl for pH adjustment at 6.8-----a proper quantity distilled water for preparation of the 1 L solution-----a proper quantity
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Sol. L: SDS solution	10% (w/v) aqueous SDS solution (1 L) SDS -----100 g distilled water for preparation of the 1 L solution-----a proper quantity
Sol. M: Stripping buffer	Stripping buffer (1 L) Sol. K -----125 mL Sol. L -----200 mL 2-mercaptoethanol ----- 7 mL distilled water-----668 mL

1) A protein-blotted PVDF membrane probed with Zn^{2+} -Phosbind Biotin and Streptavidin-conjugated HRP is soaked with Sol. C (1xTBS-T, 25 mL/5 cm²) in a Tupperware.

The membrane is gently rocked for at least 1 h. Be careful not to dry the membrane. If the membrane is dry, the membrane is soaked with methanol before this stripping treatment.

2) The membrane is soaked with Sol. M (Stripping buffer, 25 mL/5 cm²) in a Tupperware. The membrane is gently rocked for 20 min at room temperature. There is no need of heating. Confirm that the membrane does not stick to the Tupperware.

3) The membrane is soaked with Sol. C (1xTBS-T, 25 mL/5 cm²) in a Tupperware. The membrane is gently rocked for 1 h at room temperature. Wash 3 times. Confirm that the membrane does not stick to the Tupperware and be careful not to dry the membrane.

4) The membrane is blocked with an appropriate protein and then reprobed with an appropriate antibody. The chemiluminescent analysis is conducted.

5. Trouble Shooting

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1) Phosbind Biotin in Sol. E (20 μ L) is large excess amount against Streptavidin-conjugated Horseradish Peroxidase in Sol. G (1 μ L). We obtained almost the same result using smaller amount of Phosbind Biotin (e.g., 2 μ L Sol. E) and Sol. G (1 μ L) used. The user should adjust the volume of Sol. D to obtain the required sensitivity or save expenses. If the volume of Sol. E is decreased, it is no need to change the volume of the zinc(II) solution (Sol. F).

2) If the membrane is not thoroughly soaked with Sol. C, the background signal is high and spotted. Furthermore, the protein signals are not observed (i.e., white spots in the right-side figure). Confirm that the membrane does not repel Sol. C.

3) PVDF membrane is highly recommended for the Phosbind Biotin method.

6. FAQ

1) Q: What is the sensitivity level like?

A: It is at the nanogram level. Use a high-luminescence reagent such as ImmunoStar LD.

2) Q: Do we need other reagents besides this product?

A: Prepare a Streptavidin-conjugated HRP solution.

3) Q: How many times can Phosbind Biotin BTL-104 be used?

A: It depends on the frequency of use. Please refer to the following as a guide. BTL-104: 130~1300 times

4) Q: Can phosphorylated proteins be assayed?

A: You can do semi-quantitative assay based on the density of bands.

5) Q: Is it possible to determine the number of binding phosphate groups?

A: No, it isn't.

6) Q: Can I strip the antibodies of Phosbind Biotin BTL-104?

A: Yes, you can. Mix it with a solution containing 62.5 mM of Tris-HCl (pH 6.8), 2% (w/v) of SDS, and 0.1 M of 2-mercaptoethanol and shake the mixture for 15 minutes. Then, wash the mixture with

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1×TBS-T three times for 10 minutes each time.

7) Q: What kind of membrane is recommended?

A: We recommend PVDF membranes.

8) Q: Does the use of Phosbind Biotin BTL-104 require blocking?

A: No, it doesn't. Blocking causes the sensitivity to drop.

Background

Phosbind is a new type of phosphate-binding tag and functional molecule that can specifically bind phosphate ions under neutral pH (physiological pH) conditions. Phosbind is a bicyclic metal complex (1,3-bis[bis(pyridin-2-ylmethyl) amino]propan-2-olato dizinc(II) complex), which is a selective phosphate binding tag in an aqueous solution with a neutral pH, in the case of Zn^{2+} , its K_d value for phenylphosphate dianion ($PH-OPO_3^{2-}$) is 25 nM. Therefore, various methods for phosphoproteomic studies using Phosbind derivatives were developed. Here, we introduce a new application of Phosbind, which is the chemiluminescence detection of all phosphorylated proteins on electroblotted PVDF membranes using biotinylated Phosbind zinc(II) complex.

Phosbind Biotin provides a sensitive method for detecting phosphorylated proteins on PVDF membranes that requires streptomycin-conjugated horseradish peroxidase (HRP) and chemiluminescent detection reagents.

References:

- [1] Fanqiang Meng, Fengxia Lu, et al. "Acetate and auto-inducing peptide are independent triggers of quorum sensing in *Lactobacillus plantarum*." *Mol Microbiol.* 2021 Jul;116(1):298-310. PMID: 33660340
- [2] Haijing Guo, Jen-Hsuan Wei, et al. "TPX2 activation by GM130 controls astral microtubule formation and spindle orientation." *bioRxiv.* March 17, 2020.

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