PhosphoBLOCKER™ Blocking Reagent

CATALOG NUMBER: AKR-104 **STORAGE:** Room Temperature

QUANTITY AND CONCENTRATION: 200 g dry blend; 5% concentration after reconstitution in 4L

Background

Protein phosphorylation-dephosphorylation is one of the major signaling mechanisms for modulating the functional properties of proteins involved in gene expression, cell adhesion, cell cycle, cell proliferation, and differentiation. Proteins can be phosphorylated by protein kinases on specific serine, threonine, or tyrosine residues. The utilization of anti-phosphoprotein antibodies in western blotting has become a commonly used tool for signal transduction research. Unfortunately, low levels of endogenous phosphoprotein in various cell lysates often can not be detected, even with high concentrations of antibody and long exposure times. Most commercially available western blot blockers (e.g. dry milk, serum) are sufficient to block the unreacted sites on the membrane, reducing the amount of nonspecific antibody binding during the assay; however, they are not designed to preserve phosphoprotein antigens during blotting.

Cell Biolabs' PhosphoBLOCKER™ contains a proprietary formulation that provides several advantages over conventional blockers:

- Designed specifically for phosphoprotein blotting
- Enhances low level phosphoprotein signal without increasing background
- Premixed dry blend, easy to use

Methods

Freshly prepare 5% PhosphoBLOCKER™ solution in TBST or PBST. Use the 5% PhosphoBLOCKER™ solution to block the blot. When probing the blot, use the 5% PhosphoBLOCKER™ solution to dilute primary and secondary antibodies.

Notes:

- *Reconstituted PhosphoBLOCKER™ solution is only good for one week at 4ºC.*
- *The presence of dark-colored particles is a normal artifact of our manufacturing process and will not adversely affect the performance of the product. If desired, the particles may be removed following reconstitution by filtration using standard laboratory filter paper.*

Example of results

The following figures demonstrate typical titration results. One should use the data below for reference only. This data should not be used to interpret actual results.

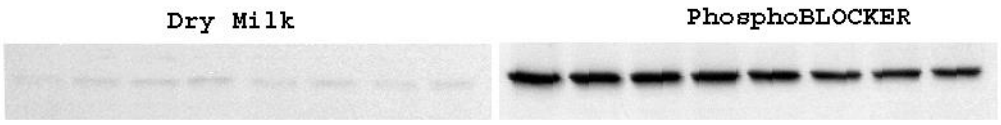

Figure 1. Western Blot of Phospho-p38 in A549 cell lysate.

Recent Product Citations

1. Tręda, C. et al. (2023). Increased EGFRvIII Epitope Accessibility after Tyrosine Kinase Inhibitor Treatment of Glioblastoma Cells Creates More Opportunities for Immunotherapy. *Int J Mol Sci*. **24**(5):4350. doi: 10.3390/ijms24054350.
2. Goddi, A. et al. (2022). Laminin-α4 negatively regulates adipocyte beiging through the suppression of AMPKα in male mice. *Endocrinology*. doi: 10.1210/endocr/bqac154.
3. Egbert, C. et al. (2022). The integration of proteome-wide PTM data with protein structural and sequence features identifies phosphorylations that mediate 14-3-3 interactions. *J Mol Biol*. doi: 10.1016/j.jmb.2022.167890.
4. Pinheiro, I. et al. (2022). A Nine-Strain Bacterial Consortium Improves Portal Hypertension and Insulin Signaling and Delays NAFLD Progression In Vivo. *Biomedicines*. **10**(5):1191. doi: 10.3390/biomedicines10051191.
5. Yamazaki, K. et al. (2022). D-allose enhances the efficacy of hydroxychloroquine against Lewis lung carcinoma cell growth by inducing autophagy. *Oncol Rep*. **47**(6):117. doi: 10.3892/or.2022.8328.
6. Yamada, H. et al. (2022). Siwi cooperates with Par-1 kinase to resolve the autoinhibitory effect of Papi for Siwi-piRISC biogenesis. *Nat Commun*. **13**(1):1518. doi: 10.1038/s41467-022-29193-9.
7. Machino, H. et al. (2022). The metabolic stress-activated checkpoint LKB1-MARK3 axis acts as a tumor suppressor in high-grade serous ovarian carcinoma. *Commun Biol*. **5**(1):39. doi: 10.1038/s42003-021-02992-4.
8. Bravo, M. et al. (2021). Synergic effect of atorvastatin and ambrisentan on sinusoidal and hemodynamic alterations in a rat model of NASH. *Dis Model Mech*. **14**(5):dmm048884. doi: 10.1242/dmm.048884.
9. Chan, T.Y. et al. (2021). TNK1 is a ubiquitin-binding and 14-3-3-regulated kinase that can be targeted to block tumor growth. *Nat Commun*. **12**(1):5337. doi: 10.1038/s41467-021-25622-3.
10. Ito, S. et al. (2021). Enoxaparin promotes functional recovery after spinal cord injury by antagonizing PTPRσ. *Exp Neurol*. **340**:113679. doi: 10.1016/j.expneurol.2021.113679.
11. Tsuchiya, M. et al. (2021). Functional analysis of isoflavones using patient-derived human colonic organoids. *Biochem Biophys Res Commun*. **542**:40-47. doi: 10.1016/j.bbrc.2021.01.021.
12. Shimazaki, R. et al. (2021). Complement factor B regulates cellular senescence and is associated with poor prognosis in pancreatic cancer. *Cell Oncol (Dordr)*. doi: 10.1007/s13402-021-00614-z.
13. Okada, R. et al. (2021). Low magnetic field promotes recombinant human BMP-2-induced bone formation and influences orientation of trabeculae and bone marrow-derived stromal cells. *Bone Rep*. doi: 10.1016/j.bonr.2021.100757.
14. Shiraishi, N. et al. (2020). Heat shock protein 90 as a molecular target for therapy in oral squamous cell carcinoma: Inhibitory effects of 17-DMAG and ganetespib on tumor cells. *Oncol Rep*. doi: 10.3892/or.2020.7873

15. Gong, Y. et al. (2020). Identification of PTPRσ-interacting proteins by proximity-labeling assay. *J Biochem*. doi: 10.1093/jb/mvaa141.
16. Kushioka, J. et al. (2020). A novel negative regulatory mechanism of Smurf2 in BMP/Smad signaling in bone. *Bone Res*. doi: 10.1038/s41413-020-00115-z.
17. Attili, I. et al. (2020). SRC and PIM1 as potential co-targets to overcome resistance in MET deregulated non-small cell lung cancer. *Transl Lung Cancer Res*. **9**(5):1810-1821. doi: 10.21037/tlcr-20-681.
18. Kushioka, J. et al. (2020). The small compound, TD-198946, protects against intervertebral degeneration by enhancing glycosaminoglycan synthesis in nucleus pulposus cells. *Sci Rep*. **10**(1):14190. doi: 10.1038/s41598-020-71193-6.
19. Orthofer, M. et al. (2020). Identification of ALK in Thinness. *Cell*. **181**(6):1246-1262.e22. doi: 10.1016/j.cell.2020.04.034.
20. Osrodek, M. et al. (2020). Insulin Reduces the Efficacy of Vemurafenib and Trametinib in Melanoma Cells. *Cancer Manag Res*. **12**:7231-7250. doi: 10.2147/CMAR.S263767.
21. Chabloz, A. et al. (2020). Salmonella-based platform for efficient delivery of functional binding proteins to the cytosol. *Commun Biol*. **3**(1):342. doi: 10.1038/s42003-020-1072-4.
22. Williams, J.J.L. et al. (2020). Investigation of Novel Cavin-1/Suppressor of Cytokine Signaling 3 (SOCS3) Interactions by Coimmunoprecipitation, Peptide Pull-Down, and Peptide Array Overlay Approaches. *Methods Mol Biol*. **2169**:105-118. doi: 10.1007/978-1-0716-0732-9_10.
23. Orthofer, M. et al. (2020). Identification of ALK in Thinness. *Cell*. S0092-8674(20)30497-9. doi: 10.1016/j.cell.2020.04.034.
24. Zeng, X. et al. (2020). Effect of Low Dose of Berberine on the Radioresistance of Cervical Cancer Cells via a PI3K/HIF-1 Pathway under Nutrient Deprived Conditions. *Int J Radiat Biol*. doi: 10.1080/09553002.2020.1770358.
25. Koizumi, R. et al. (2020). Relationship between hemodynamic alteration and sympathetic nerve activation following a single oral dose of cinnamtannin A2. *Free Radic Res*. doi: 10.1080/10715762.2020.1759805.
26. Yamaguchi-Ueda, K. et al. (2019). Combination of ions promotes cell migration via extracellular signal-regulated kinase 1/2 signaling pathway in human gingival fibroblasts. *Molecular Medicine Reports*. doi: 10.3892/mmr.2019.10141.
27. Nihei, Y. et al. (2019). Poly-glycine-alanine exacerbates C9orf72 repeat expansion-mediated DNA damage via sequestration of phosphorylated ATM and loss of nuclear hnRNPA3. *Acta Neuropathol*. doi: 10.1007/s00401-019-02082-0.
28. Taniguchi, K. et al. (2019). α-Aminoisobutyric acid-containing amphipathic helical peptide-cyclic RGD conjugation as a potential drug delivery system for microRNA replacement therapy in vitro. *Mol Pharm*. doi: 10.1021/acs.molpharmaceut.9b00680.
29. Serrano-Regal, M.P. et al. (2019). Oligodendrocyte Differentiation and Myelination Is Potentiated via GABAB Receptor Activation. *Neuroscience*. pii: S0306-4522(19)30488-9. doi: 10.1016/j.neuroscience.2019.07.014.
30. Czyz, M. et al. (2019). Plasticity of Drug-Naïve and Vemurafenib- or Trametinib-Resistant Melanoma Cells in Execution of Differentiation/Pigmentation Program. *Journal of Oncology*. **2019**:1697913. doi: 10.1155/2019/1697913.

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