Phospho-NPM (Ser4) (A1) rabbit mAb

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Applications	Detection	Clonality	Isotype
Flow Cytometry,WB	Anti-Rabbit IgG	Monoclonal	Rabbit IgGk

Format: Unconjugated

Cross Reactivity: Predicted to work with mouse, rat and other homologues.

Formulation: 1X PBS, 0.02% NaN3, 50% Glycerol, 0.1% BSA

Preparation: Protein A+G

Reactivity: Human

Recommended

References:

Usage:

 $1\mu g/mL$ – $0.001\mu g/mL$. It is recommended that the reagent be titrated for optimal performance for each application. See product image legends for additional

information.

Immunogen: A synthetic phospho-peptide corresponding to residues surrounding Ser4 of human

phospho-NPM

Description: Nucleophosmin (NPM1) also known as B23, is a novel substrate for G-protein-

coupled receptor kinases (GRKs). NPM1 belongs to the nucleoplasmin family of proteins, made of the nucleophosmin, nucleoplasmin (NPM2), and NPM3 (1). GRKs phosphorylate, desensitize, and traffic GPCRs. In addition, they phosphorylate non-receptors substrates, thus potentially regulating a variety of cellular processes (2). GRK5 and GRK6 are found in nucleus and may be regulated by Gq signaling (3,4). GRK5 has been shown to phosphorylate NPM1. GRK5 phosphorylate NPM1 on Ser4, and Thr199 in vitro. Ser4 phosphorylation is also targeted by PLK1 (5). It is of interest that GRK5 phosphorylation of NPM1 may confer resistance to cell death mediated by PLK1 inhibition. Phosphorylation of NPM1 at Ser4 by PLK1 is involved in mitotic spindle formation (5). Whereas phosphorylation of NPM1 at Thr199 by cyclin-dependent kinase 1 is involved in regulating the centrosome (6), whereas the phosphorylation of both Ser4 and Thr199 controls centrosome duplication (7). NPM1 is involved in a variety of functions, including the regulation of centrosomal duplication, the cell cycle, mitosis, apoptosis, and RNA and DNA replication. It also

serves as a chaperone for proteins such as histone. NPM1 is overexpressed in number of cancers and thus, is a potential target for cancer therapeutics (8).

1. Frehlick LJ, et al., 2007, Bioessays, 29:49-59.

2. Gurevich EV., et al., 2012, Pharmacol Ther, 133:40-69

3. Martini JS., et al., 2008, Proc Natl Aca Sci USA, 105:12457-12462.

4. Johnson LR., et al., 2004, Mol Cell Biol, 24:10169-10179.

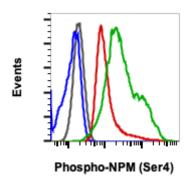
5. Zhang H., eta., 2004, J Biol Chem, 279:35726-35734.

6. Okuwaki M., 2008, J Biochem., 143:441-8.

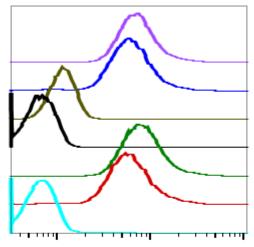
7. Michal AM., et al., 2012, J Biol. Chem., 287:6928-40.

8. Grisendi S., et al., 2006, Nat. Rev. Cancer 6:493-505.



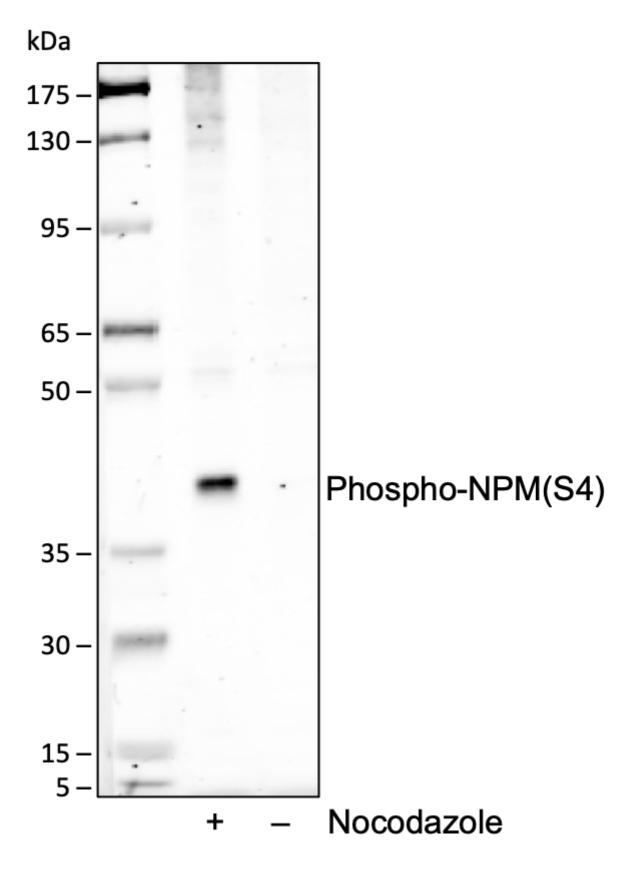


Flow cytometric analysis of C6 cells untreated (red) or treated with nocodazole (green) using Phospho-NPM (Ser4) (A1) rabbit mAb NPMS4-A1 #2466 at 0.01 ug/mL, or concentration-matched rabbit (G9) mAb IgG Isotype Control #2141 for cells untreated (black) or treated with nocodazole (blue).



\$WELLIOD	Treatment	Peptide Block	Median : BL1-A
A1 0.01	PV	Non-Phos	7002
A1 0.01	imatinib	Non-Phos	5916
A1 0.01	PV	Phos	1140
A1 0.01	imatinib	Phos	658
A1 0.01	PV	-	7909
A1 0.01	imatinib	-	5791
2'AB	imatinib	-	656

Peptide blocking flow cytometric analysis of K562 cells secondary antibody only negative control (light blue) or treated with imatinib (red) or treated with pervanadate (green) or treated with imatinib and blocked with phospho-peptide (black) or treated with pervanadate and blocked with phospho-peptide (gold) or treated with imatinib and blocked by non-phospho-peptide (dark blue) or treated with pervanadate and block with non-phospho-peptide (purple) using Phospho-NPM (Ser4) antibody NPMS4-A1 at 0.01 ug/mL, Cat#2466.



Western blot analysis of extract from HeLa cells untreated or treated with no codazole using Phospho-NPM (Ser4) antibody NPMS4-A1 at 01 ug/mL, Cat #2466