

# Phospho-NPM (Thr199) Antibody



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**For Research Use Only. Not For Use In Diagnostic Procedures.**

Entrez-Gene ID #4869  
UniProt ID #P06748

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IHC-P, IF-IC Endogenous	H, M, R	38	Rabbit**

**Background:** Nucleophosmin (NPM; also known as B23, numatrin or NO38) is an abundant phosphoprotein primarily found in nucleoli. It has been implicated in several distinct cellular functions, including assembly and transport of ribosomes, cytoplasmic/nuclear trafficking, regulation of DNA polymerase alpha activity, centrosome duplication and molecular chaperoning activities (1,2). The NPM gene is also known for its fusion with the anaplastic lymphoma kinase (ALK) receptor tyrosine kinase. The NPM portion contributes to transformation by providing a dimerization domain, which results in activation of the fused kinase (3,4).

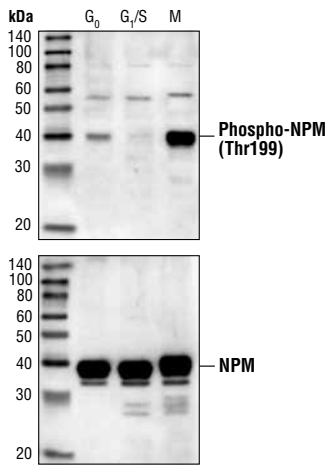
NPM associates with unduplicated centrosomes and is a direct substrate of Cdk2-cyclin E in centrosome duplication (4). Upon phosphorylation at Thr199 by Cdk2-cyclin E, NPM dissociates from centrosomes, and this dissociation is a prerequisite step for centrosome to initiate duplication (5).

**Specificity/Sensitivity:** Phospho-NPM (Thr199) Antibody detects endogenous levels of NPM only when phosphorylated at threonine 199.

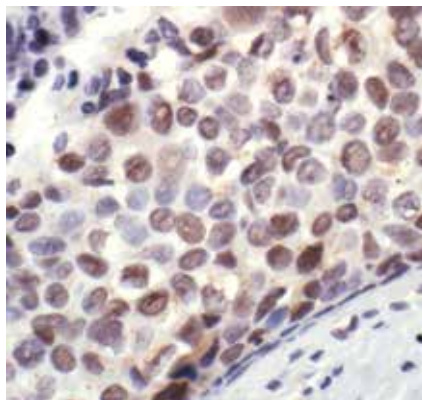
**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Thr199 of human NPM. Antibodies are purified by protein A and peptide affinity chromatography.

**Background References:**

- (1) Okuda, M. et al. (2000) *Cell* 103, 127-140.
- (2) Takemura, M. et al. (1999) *J. Biochem. (Tokyo)* 125, 904-909.
- (3) Morris, S. W. et al. (1994) *Science* 263, 1281-1284.
- (4) Bischof, D. et al. (1997) *Mol. Cell. Biol.* 17, 2312-2325.
- (5) Tokuyama, Y. et al. (2001) *J. Biol. Chem.* 276, 21529-21537.



Western blot analysis of extracts from HeLa cells synchronized at various stages of the cell cycle, using Phospho-NPM (Thr199) Antibody (upper) or NPM Antibody #3542 (lower).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear localization, using Phospho-NPM (Thr199) Antibody.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

\*Species cross-reactivity is determined by western blot.

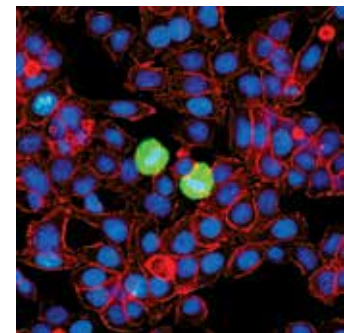
\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:200
Unmasking buffer:	Citrate
Antibody diluent:	TBST-5%NGS
Immunofluorescence (IF-IC)	1:400

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

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Confocal immunofluorescent analysis of HT-29 cells, untreated (left) or λ-phosphatase-treated (right), using Phospho-NPM (Thr199) Antibody (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.**

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**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.