

p-nestin (Thr 1583): sc-33880

BACKGROUND

Nestin is a major intermediate filament (IF) protein of embryonic central nervous system progenitor cells. Nestin is also a component of the dynamic IF network during muscle development where it polymerizes with desmin and vimentin. Nestin co-assembles with vimentin or α -internexin and forms heterodimer coiled-coil molecules which then further assemble into 10 nm IFs. Deletion of the IF consensus rod domain in nestin alters nestin localization in CNS precursor cells and radial glial cells *in vivo*. Nestin is a marker for neuroepithelial stem cells, glioma cells, and tumor endothelial cells during rapid growth. During axon elongation of differentiation neurons, nestin localizes to the growth cones and may play a role in growth cone guidance. In the rat adrenal gland, nestin is expressed by the zona fasciculata and the zona reticularis. Nestin is also expressed by dermatomal cells and by myoblasts during the earliest stages of myogenesis. Thr 316 is a Cdc2-specific phosphorylation site in Nestin. Phosphorylation at this site is increased during mitosis and is used as a marker for Cdk5-specific phosphorylation. Cdk5 and Cdc2 induces phosphorylation at both residues Thr 316 and Thr 1583.

REFERENCES

- Lendahl, U., et al. 1990. CNS stem cells express a new class of intermediate filament protein. *Cell* 60: 585-595.
- Sejersen, T., et al. 1993. Transient expression of the intermediate filament nestin during skeletal muscle development. *J. Cell Sci.* 106: 1291-1300.
- Kachinsky, A.M., et al. 1994. Myogenesis and the intermediate filament protein, nestin. *Dev. Biol.* 165: 216-228.
- Marvin, M.J., et al. 1998. A ROD end deletion in the intermediate filament protein nestin alters its subcellular localization in neuroepithelial cells of transgenic mice. *J. Cell Sci.* 111: 1951-1961.
- Steinert, P.M., et al. 1999. A high molecular weight intermediate filament-associated protein in BHK-21 cells is nestin, a type VI intermediate filament protein. Limited co-assembly *in vitro* to form heteropolymers with type III Vimentin and type IV α -internexin. *J. Biol. Chem.* 274: 9881-9890.

CHROMOSOMAL LOCATION

Genetic locus: NES (human) mapping to 1q23.1; Nes (mouse) mapping to 3 F1.

SOURCE

p-nestin (Thr 1583) is a rabbit polyclonal antibody raised against a short amino acid sequence containing Thr 1583 phosphorylated nestin of rat origin.

PRODUCT

Each vial contains 200 μ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-33880 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

p-nestin (Thr 1583) is recommended for detection of Thr 1583 phosphorylated nestin of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-nestin (Thr 1583) is also recommended for detection of correspondingly phosphorylated nestin in additional species, including equine, canine and porcine.

Suitable for use as control antibody for nestin siRNA (h): sc-36032, nestin siRNA (m): sc-36033, nestin shRNA Plasmid (h): sc-36032-SH, nestin shRNA Plasmid (m): sc-36033-SH, nestin shRNA (h) Lentiviral Particles: sc-36032-V and nestin shRNA (m) Lentiviral Particles: sc-36033-V.

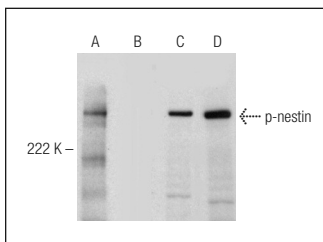
Molecular Weight of p-nestin: 190-200 kDa.

Positive Controls: Mouse embryonic brain tissue extract.

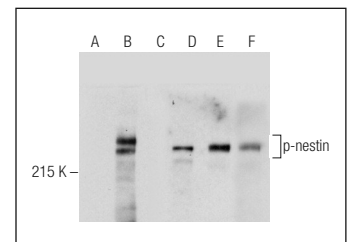
RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



Western blot analysis of nestin phosphorylation in untreated (A, D), induction cocktail (sc-362324) treated (B, E) and induction cocktail (sc-362324) and lambda protein phosphatase (sc-200312A) treated (C, F) SH-SY5Y whole cell lysates. Antibodies tested include p-nestin (Thr 1495): sc-33880 (A, B, C) and nestin (C-20): sc-21247 (D, E, F).



Western blot analysis of nestin phosphorylation in untreated (A, D), induction cocktail (sc-362324) treated (B, E) and induction cocktail (sc-362324) and lambda protein phosphatase (sc-200312A) treated (C, F) SH-SY5Y whole cell lysates. Antibodies tested include p-nestin (Thr 1495): sc-33880 (A, B, C) and nestin (C-20): sc-21247 (D, E, F).

SELECT PRODUCT CITATIONS

- Sahlgren, C.M., et al. 2003. Cdk5 regulates the organization of Nestin and its association with p35. *Mol. Cell. Biol.* 23: 5090-5106.

RESEARCH USE

For research use only, not for use in diagnostic procedures.