

Cot (H-212): sc-9167

BACKGROUND

The role of mitogen-activated protein kinases (MAPKs) in cell signaling pathways is well established. The rat gene *Tpl-2*, for tumor progression locus 2, and the human and mouse homologues *c-Cot*, for cancer osaka thyroid oncogene, encode a proto-oncogene serine/threonine protein kinase that was shown to play a role in the functional activation of the MAP kinase pathway. Overexpression of Cot induces MAP kinase activation in COS-1 and NIH/3T3 cells. Cot-mediated activation of MAP kinase is inhibited by both Ras N17, a dominant negative mutant of c-H-Ras, and Raf-1s621A, a dominant negative mutant of Raf-1, suggesting that Cot functions upstream of Ras and Raf-1. Other studies have shown that a kinase-negative, dominant negative mutant of Cot partially blocks Ras or Raf-1-induced MAP kinase activation, arguing that Cot functions downstream of Ras and Raf-1. To explain these contrasting findings, it has been suggested that Cot, Ras and Raf-1 may form a multimeric complex that phosphorylates MEK-1. Cot has also been shown to be implicated in T lymphocyte activation. Two forms of Cot, are produced by alternative initiation of translation.

REFERENCES

1. Haubruk, H., et al. 1991. Ras p21: effects and regulation. *Biochim. Biophys. Acta* 1072: 215-229.
2. Roberts, T.M. 1992. A signal chain of events. *Nature* 360: 534-535.
3. Nishida, E., et al. 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem. Sci.* 18: 128-131.
4. Fabian, J.R, et al. 1993. Requirement for Raf and MAP kinase function during the meiotic maturation of *Xenopus* oocytes. *J. Cell Biol.* 122: 645-652.
5. Aoki, M., et al. 1993. The human cot proto-oncogene encodes two protein serine/threonine kinases with different transforming activities by alternative initiation of translation. *J. Biol. Chem.* 268: 22723-22732.
6. Patriotis, C., et al. 1994. *Tpl-2* acts in concert with Ras and Raf-1 to activate mitogen-activated protein kinase. *Proc. Natl. Acad. Sci. USA* 91: 9755-9759.
7. Ballester, A., et al. 1998. Cot kinase activates tumor necrosis factor- α gene expression in a cyclosporin A-resistant manner. *J. Biol. Chem.* 273: 14099-14106.

CHROMOSOMAL LOCATION

Genetic locus: MAP3K8 (human) mapping to 10p11.23; Map3k8 (mouse) mapping to 18 A1.

SOURCE

Cot (H-212) is a rabbit polyclonal antibody raised against amino acids 256-467 mapping at the C-terminus of Cot of human origin.

PRODUCT

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

APPLICATIONS

Cot (H-212) is recommended for detection of Cot 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).

Cot (H-212) is also recommended for detection of Cot in additional species, including equine, canine, bovine, porcine and avian.

Suitable for use as control antibody for Cot siRNA (h): sc-35095, Cot siRNA (m): sc-35096, Cot shRNA Plasmid (h): sc-35095-SH, Cot shRNA Plasmid (m): sc-35096-SH, Cot shRNA (h) Lentiviral Particles: sc-35095-V and Cot shRNA (m) Lentiviral Particles: sc-35096-V.

Molecular Weight of Cot: 52/58 kDa.

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.

SELECT PRODUCT CITATIONS

1. Wu, B., et al. 2008. Proteomics analysis of immunoprecipitated proteins associated with the oncogenic kinase cot. *Mol. Cells* 25: 43-49.

STORAGE

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

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.



Try **Cot (H-7): sc-373677**, our highly recommended monoclonal alternative to Cot (H-212).