Qty: 100 µg/200 µl
Mouse anti-RanGAP-1
Catalog No. 33-0800
Lot No.

Mouse anti-RanGAP-1

FORM
This monoclonal antibody is highly purified from mouse ascites by protein A-affinity chromatography. It is supplied as a 200 µl aliquot at a concentration of 0.5 mg/ml in phosphate buffered saline, pH 7.4, containing 0.1% sodium azide.

CLONE: 19C7 **ISOTYPE:** Mouse IgG_1-kappa

IMMUNOGEN
A fusion protein consisting of the first 203 amino-terminal residues of the mouse RanGAP-1 protein.

SPECIFICITY
This monoclonal antibody can be used to specifically detect both the unmodified (~ 70 kDa) form and the GMP1 (SUMO-1) conjugated (~90 kDa) form of the RanGAP-1 protein. The ~70 kDa form of RanGAP-1 is cytoplasmic, whereas the ~90 kDa form is associated predominately with the nuclear envelope where it is localized to the nuclear pore complexes (NPCs).

REACTIVITY
Species Reactivity: Human and rat.
Lysates Tested: Rat liver nuclear envelopes, total lysates derived from NIH 3T3 and HeLa cells.

USAGE
The concentrations below are only starting recommendations. Optimal concentrations of this antibody should be determined by the investigator for each specific application.

ELISA:	0.1-1 µg/ml
Western Blotting[8]:	0.1- 1 µg/ml
Immunofluorescence[8]:	1 µg/ml

Note: For Immunofluorescence staining, see the methods section of reference # 8.

STORAGE
Store at 2-8°C for up to one month. Store at -20°C for long term storage. Do not repeatedly freeze and thaw.

BACKGROUND
Transport of macromolecules between the nucleus and cytoplasm occurs bi-directionally and is mediated by nuclear pore complexes (NPCs). NPCs are large supermolecular structures that span the nuclear envelope. Although ions, metabolites, and small macromolecules can passively diffuse through the NPC, most larger macromolecules such as proteins and ribonucleoproteins (RNPs) must be transported across the NPC by specific signal- and energy -dependent mechanisms.

To date, the import of proteins into the nucleus has been studied in great detail and is considered to be the most well characterized aspect of nucleocytoplasmic transport. Nuclear import is specified by nuclear targeting or nuclear localization sequences (NLSs) in the imported protein. These NLSs typically consist of short stretches of basic amino acids arranged in either a continuous or bipartite motif. A variety of studies based primarily on in vitro import assays have led to the

(cont'd)

identification of five conserved soluble proteins that are involved in the import process: the NLS receptor (importin α, karyopherin α, Srp1p), p97 (importin β, karyopherin β, Kap95), hsp/hsc 70, NTF2 (p10, pp15), and the small Ras-like GTPase Ran. In addition to Ran, at least one other GTPase may also be involved in import. Recognition of NLS containing substrates in the cytoplasm is mediated by the α subunit (importin α, karyopherin α, Srp1p) of the α-β heterodimeric NLS receptor. Docking of this trimeric complex to the NPC is then mediated by the β subunit (importin β, karyopherin β, Kap95). The GTPase Ran and the Ran interactive protein NTF2 (p10, pp15) appear to mediate the dissociation of these substrate/receptor complexes as they dock along the substrate/receptor docking sites.

Ran is required for the bi-directional transport of proteins and RNPs across the NPC. Ran is thought to operate in much the same fashion as other Ras-like GTPases acting as a molecular switch by cycling between the GTP bound and GDP bound forms. Based on the results of several studies, Ran appears to be converted into its GTP-bound form in the nucleus and hydrolyzed into its GDP-bound form in the cytoplasm. Nucleotide exchange by Ran is catalyzed by the GTP exchange factor RCC1, and GTP hydrolysis is catalyzed by the GTPase-activating protein RanGAP1. RCC1 is a nuclear chromatin-associated protein which was initially identified as a factor involved in the control of specific mitotic events. RanGAP1 was initially identified in S. cerevisiae as RNA1-1, a mutant defective in RNA production, processing, and nuclear export. Two other proteins which bind Ran-GTP and possibly affect GTP hydrolysis include RanBP1 and RanBP2 (Nup 358).

The mammalian RanGap-1 protein was purified from HeLa cells as a 65-70 kDa homodimer, and recent studies have shown that RanGAP1 is highly concentrated at the cytoplasmic periphery of the NPC where it associates with RanBP2. This interaction requires the RanGap1 protein to be conjugated to a ~17 kDa ubiquitin-like protein termed GMP1 (Gap modifying protein 1) or SUMO-1 (small ubiquitin-related modifier). The unmodified 70 kDa form of RanGAP1 is exclusively cytoplasmic, whereas the 90-kDa modified from of RanGap1 associates with the cytoplasmic fibers of the NPC. Therefore, the ubiquitin-like GMP-1 (SUMO-1) protein functions in a novel way by modulating the partitioning of RanGAP1 between the cytoplasm and NPC.

REFERENCES

Reviews:

1. Gorlich, D., and Mattaj, I.W. Science 271: 1513-1518.
2. Melichoir, F., and Gerace, L. (1995) Curr. Opin. Cell. Biol. 7:310-318.
3. Sweet, D. J., and Gerace, L. (1995) Trends Cell Biol. 5:444-447.

Primary References:

4. Bischoff, F.R., et al. (1994) Proc. Natl. Acad. Sci. USA 91:2587-2591.
5. Bischoff, F.R., et al. (1995) Proc. Natl. Acad. Sci. USA 92:1749-1753.
6. Bischoff, F.R. et al. (1995) EMBO J. 14:705-715.
7. DeGregori, J. et al. (1994) Genes Dev. 8:265-276.
8. Matunis, M.J. et al. (1996) J. Cell Biol. 135:1457-1470.
9. Mahajan, R. et al. (1997) Cell 88:97-107.

RELATED PRODUCTS

Primary antibodies	***Clone/PAD***	***Cat. No.***
Mouse Anti-eNOS	eNOS-9D10	33-4600
Mouse Anti-eNOS	eNOS-6C6	33-4500
HRP-Mouse Anti-eNOS	eNOS-6C6	33-4520
Rabbit Anti-iNos (human specific)	Polyclonal	61-7700
Immunoassay reagents	***Conjugate***	***Cat. No.***
Goat anti-Mouse IgG (H+L) (ZyMAX™ Grade)	Purified	81-6500
	FITC	81-6511
	TRITC	81-6514
	Cy™3	81-6515
	Cy™5	81-6516
	HRP	81-6520
	AP	81-6522
	Biotin	81-6540
Protein A	Sepharose® 4B	10-1041
rec-Protein G	Sepharose® 4B	10-1241

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