

Qty: 50 µg/100 µl Rat anti-v-H-ras Catalog No. 33-7200 Lot No.

Rat anti-v-H-ras

FORM

This monoclonal antibody is supplied as a 100 µl aliquot of purified antibody, at a concentration of 0.5 mg/ml in PBS, pH 7.4, containing 0.1% sodium azide.

CLONE: Y13-259⁽¹⁾ **ISOTYPE:** Rat IgG₁-kappa

IMMUNOGEN: Recombinant p21 ras protein.

SPECIFICITY

This antibody reacts with the phosphorylated and non-phosphorylated forms of rat, human and mouse ras. It does not crossreact with the rap protein. The epitope recognized by anti-ras clone Y13-259 is comprised by amino acids 62-76 of human ras.

USAGE

Working concentrations for specific applications should be determined by the investigator. Appropriate dilutions will be affected by several factors, including secondary antibody affinity, antigen concentration, sensitivity of detection method, temperature and length of incubations, etc. The suitability of this antibody for applications other than those listed below has not been tested. We recommend the following as starting points for this product.

Application	Amount	Positive Control
Immunofluorescence	2.5 µg/ml	SW480 or
		Y1 cells
Immunohistochemistry (frozen)	5 µg/ml	normal skin
Immunohistochemistry (staining of formalin fixed, paraffin embedded tissue requires pepsin pretreatment. Use saponin to permeablize)	1-5 μg/ml	normal skin
Immunoprecipitation*	1 µg/sample	SW480 or
(autoradiographic detection)		Y1 cells
Western Blotting	2.5 µg/ml	A431, HeLa
(chemiluminescence detection)		or ML-1 cells
Neutralization of H-, K- and N-ras		
p21 biochemical activity**		

* See references 1 and 3. For immunopreciptiation, use Rb x-Rat IgG plus Protein A or Gt x Rat IgG plus Protein G. Protein A alone will not immunoprecipitate rat IgG.Doublets corresponding to farnesylated- and non-farnasylated-ras may be observed. Detects both phosphorylated and non-phosphorylated forms of v-H-ras and v-K-ras p21's as well as the p21 translational products of the H-, K- and N-ras human genes. ** The antibody neutralizes the biological and biochemical activities of by binding to residues E63, S65, A66, M67, Q70 and R73.⁽²⁾

STORAGE

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Store at 2-8°C for up to one month. Store at -20°C for long term storage. Avoid repeated freezing and thawing.

(cont'd)

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BACKGROUND

v-H-*ras*, K-*ras* and N-*ras* are 21 kDa phosphoproteins originally isolated based on their oncogenic properties and later found in all eukaryotic cells. *Ras* proteins belong to the Ras superfamily of monomeric GTPases which also includes the Rho, Rac, and Rab subfamilies. *Ras* proteins are anchored to the cytoplasmic face of plasma membranes where they function in switch-like fashion to relay signals from receptor tyrosine kinases to downstream effector molecules.

Ras activation is regulated by the opposing effects of the activating guanine nucleotide exchange factors (GNEFs) that exchange ras-bound GDT with GTP and the inactivating GTPase accelerating proteins (GAPs). Ras activation of the MAP kinase signaling cascade is though to occur via direct interaction of ras with the serine/thronine MAP-kinase-kinase raf.

REFERENCES

<u>Cited</u>

1. Furth, M.E., et al., *J. Virol.* 43:294-304 (1982).

2. Signal, I.S., et al., Proc. Natl. Acad. Sci. USA 84:4725-4729 (1986).

3. Furth, M.E., et al., Oncogene 1:47-58 (1987).

<u>General</u>

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Ellis, R.W., et al., *Nature* 292:506-511 (1981). Shih, T.Y., et al., *Nature*, 287: 686-691 (1980). Shimizu, K., et al., *Proc. Natl. Acad. Sci.* USA 80:2112-2116 (1983). Taparowsky, E., et al., *Cell* 34:581-586 (1983).

RELATED PRODUCTS

Product	Conjugate	Cat. No.
Goat anti-Rat IgG (H+L)	Purified	62-9500
	FITC	62-9511
	TRITC	62-9514
	Су™З	62-9515
	Cy™5	62-6516
	HRP	62-9520
	AP	62-9522
	Biotin	62-9540
	Sepharose [®] 4B	62-9541
	-	
Mouse anti-Rat IgG (H+L)	Purified	80-9500
(Polyclonal)	FITC	80-9511
	HRP	80-9520
	Biotin	80-9540
Rabbit anti-Rat IgG (H+L)	Purified	61-9500
rec-Protein G	Sepharose [®] 4B	10-12/1
Protoin A	Sepheroze [®] 4P 10.1041	
FIULEIIIA	Sephalose 4D	10-1041

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