

Background: The human *ras* gene family consists of three identified members, H, K and N-*ras*, encoding proteins of 188-189 amino acids and 21,000 (p21) molecular weight (1,2). Human H- and K-*ras* are the homologues of v-H- and v-K-*ras* sequences originally isolated from Harvey and Kirsten strains of rat sarcoma viruses (3,4). Normal human cellular *ras* genes can be activated to oncogenes by mutations occurring in codons 12, 13 and 61; such mutated, activated and transforming *ras* genes have been identified and isolated from human tumors and cultured tumor cells (for review see 5). Although the expression patterns of *ras* proto-oncogene proteins in normal human tissues are known (6), similar information for activated *ras* oncogene encoded p21's and their relevance to human disease diagnosis and prognosis is still emerging (7, 8, 9).

Origin: Clone F235-1.7.1 is a mouse monoclonal antibody generated by immunizing BALB/c mice with recombinant p21 protein and fusing with P3X63 Ag8.653 myeloma cells.

Characteristics:

 Isotype: IgG₁κ

Epitope: within residues 54-188

Species	human	mouse	rat	other
Reactivity	Y	Y	Y	NT

legend: Y=yes NT=not tested

Applications:

Immuno-Precipitation*	amount	label	positive control
	5 µg per reaction	³⁵ S-Met	ras 1 cells

Frozen Sections	amount	positive control	negative control
	5 µg/mL	normal skin	<i>trpE</i> (Ab-1)

Paraffin Sections	amount	detergent	enzyme	positive control	negative control
	5 µg/mL	saponin	pepsin	normal skin	<i>trpE</i> (Ab-1)

Western Blotting*	amount	chemi-luminescent	colori-metric	positive control
	10 µg/mL	NT	Y	ras 1 cells

Immuno-fluorescence	amount	positive control
	2.5 µg/mL	ras 1 cells

legend: Y=yes NT=not tested

*See Comments

How Supplied: 100 µg or 200 µg (Cat# OP23) of purified antibody in 1.0 mL of 0.05 M sodium phosphate buffer containing 0.1% sodium azide and 0.2% gelatin; or 100 µg (Cat# OP23L) purified antibody lyophilized from a volatile buffer with 100 µg of BSA. We recommend resuspending the lyophilized antibody with sterile phosphate buffered saline (PBS), pH 7.4, or sterile 20 mM Tris-saline (20 mM Tris containing 0.15 M NaCl), pH 7.4, to yield a final concentration of 100 µg/mL; product will be more stable if 0.1% sodium azide is included (do not add azide if antibody is to be used with viable cells). Lyophilized antibody should be resuspended at 4°C with occasional gentle mixing for at least two hours.

Storage: Store Cat# OP23 (in solution) at 4°C; do not freeze. Store Cat# OP23L (lyophilized) at 4°C until reconstituted, then store in aliquots at -20°C or at 4°C with 0.1% azide; freezing of aliquots is best for storage of reconstituted product for longer than a month, but repetitive freezing and thawing should be avoided. If stored under proper conditions, product guaranteed until expiration date stated.

Comments: For immunoprecipitation, use 5 µg Cat# OP23 per sample with 45 µL protein G plus agarose. The level of expression of p21^{ras} is variable in different tissues. For this reason, we recommend a concentration step prior to western blot analysis to obtain optimal results. A doublet may be seen due to farnesylation. c-H-ras (Ab-1) will react to C-H-ras and, weakly, to v-H-ras; it does not detect either c-K-ras or c-N-ras p21s under the conditions tested. Purified p21 ras proteins are also available for western blotting standards. Suggested starting concentrations are provided. Antibodies should be titrated for optimal results in individual systems.

References:

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