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c-K-*ras* (Ab-1) — Cat# OP24, OP24L

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 p21s and their relevance to human disease diagnosis and prognosis is still emerging (7,8,9). K-*ras* mutations have been identified in several cancers to date including lung and colorectal tumors (10, 11).

Origin: Clone 234-4.2 is a mouse monoclonal antibody generated by immunizing BALB/c mice with recombinant p21 protein and fusing with Ag8.653 myeloma cells.

Characteristics:

Isotype: $IgG_{2a}\kappa$
Epitope: within amino acid residues 54-189

Species Reactivity

human	mouse	rat	other
Y	Y	Y	NT

legend: Y=yes N=no NT=not tested

Applications:

Frozen Sections

amount	positive control
10 µg/mL	normal skin

Immuno-precipitation

amount	label	positive control
see comments	^{35}S-Met	Y1 and SW 480 cells

Western Blotting

amount	chemi-luminescent	colori-metric	positive control
see comments	Y	Y	Y1 and SW 480 cells

Paraffin Sections

amount	detergent	enzyme	positive control
10 µg/mL	saponin	pepsin	normal skin

legend: Y=yes N=no

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How Supplied: 100 µg or 200 µg (Cat# OP24) 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# OP24L) 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. Lyophilized antibodies should be resuspended at 4°C with occasional gentle mixing for at least two hours.

Storage: Store Cat# OP24 (in solution) at 4°C; do not freeze. Store Cat# OP24L (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 long term storage of reconstituted product; repetitive freezing and thawing should be avoided. If stored under proper conditions, product guaranteed until expiration date stated.

Comments: c-K-*ras* (Ab-1) reacts with c- and v-K-*ras* p21, but not with c-H-*ras* or c-N-*ras* p21s. The level of expression of p21 *ras* is variable in different tissues. For this reason we recommend a concentration step prior to western blotting at 5 µg/mL with 1% gelatin (see ref. 12 for complete protocol). For immunoprecipitation, use 5 µg OP24 per sample with 45 µL protein A agarose. Suggested starting concentrations are provided. Antibodies should be titrated for optimal results in individual systems.

References:

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