Ras-related C3 botulinum toxin substrate 1

UniProt
Function: Isoform B has an accelerated GEF-independent GDP/GTP exchange and an impaired GTP hydrolysis, which is restored partially by GTPase-activating proteins. It is able to bind to the GTPase-binding domain of PAK but not full-length PAK in a GTP-dependent manner, suggesting that the insertion does not completely abolish effector interaction.
Subcellular location: Cell membrane; Lipid-anchor; Cytoplasmic side (By similarity). Melanosome. NOTE: Inner surface of plasma membrane possibly with attachment requiring prenylation of the C-terminal cysteine (By similarity). Identified by mass spectrometry in melanosome fractions from stage I to stage IV.
Tissue specificity: Isoform B is predominantly identified in skin and epithelial tissues from the intestinal tract. Its expression is elevated in colorectal tumors at various stages of neoplastic progression, as compared to their respective adjacent tissues.

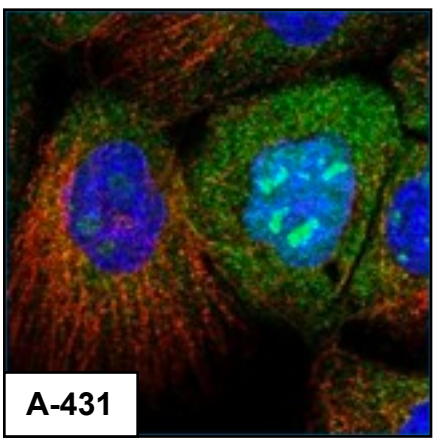
Two antibodies: RAC1-1 and RAC1-2 were tested and both antibodies were approved for IHC. RAC1-1 was selected for full protein profiling.

RAC1-1 (CAB035994)

Immunohistochemistry

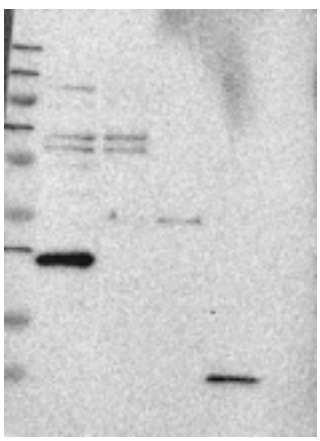
IHC protocol: HIER pH 6, Dilution 1:35
IHC test staining: Cytoplasmic (slightly granular) staining mainly in non malignant intestine, colorectal cancer and some other cancers.

IHC Annotators comments
Moderate cytoplasmic positivity was displayed in respiratory epithelium, gastrointestinal glands, urothelium, fallopian tube, placental decidual cells and bone marrow poietic cells. Many colorectal, urothelial, renal, gastric, pancreatic and liver cancers along with a few malignant melanomas, lung and skin cancers displayed weak to moderate immunoreactivity. Remaining normal and malignant tissues were generally negative.



Immunofluorescence

IF Overlay: antibody (green), anti-tubuline (red) and DAPI (blue)
IF Localisation: Staining of nuclei and cytoplasm in all three cell lines with additional staining of nucleoli in A-431. The staining of nuclei/nucleoli in A-431 and U-2 OS varies in intensity between cells.
IF Validation: Subcellular localization supported by literature.



Western blot

WB Size markers (kDa): 230, 130, 95, 72, 56, 36, 28, 17, 11
WB Lanes: Marker(1), RT-4(2), U251 MG(3), Plasma(4), Liver(5), Tonsil(6)
WB Target weight (kDa): 23, 21, 17, 16
WB Validation: Supportive (Band of predicted size in kDa (+/-20%) with additional bands present)