

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat Raf-1 at 74 kDa in Western blots.
Source	Monoclonal Mouse IgG _{2A} Clone # 563002
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Raf-1 Asn189-Thr353 Accession # P04049
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

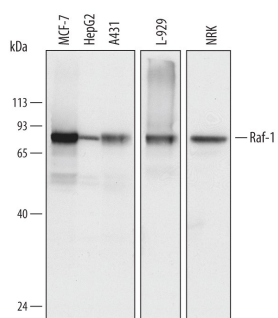
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below

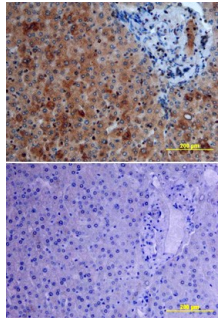
DATA

Western Blot



Detection of Human, Mouse, and Rat Raf-1 by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line, HepG2 human hepatocellular carcinoma cell line, A431 human epithelial carcinoma cell line, L-929 mouse fibroblast cell line, and NRK rat normal kidney cell line. PVDF Membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse/Rat Raf-1 Monoclonal Antibody (Catalog # MAB4540) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Raf-1 at approximately 74 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.

Immunohistochemistry



Raf-1 in Human Liver. Raf-1 was detected in immersion fixed paraffin-embedded sections of human liver array using Mouse Anti-Human/Mouse/Rat Raf-1 Monoclonal Antibody (Catalog # MAB4540) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

The Raf serine/threonine kinases are effectors of Ras that function as MAP3Ks in the ERK phosphorylation cascade. Mammals express three Raf proteins: A-Raf, B-Raf, and Raf-1, also known as C-Raf. Human Raf-1 contains three distinct regions; an N-terminal RBD (Ras-binding domain) (aa 56 - 131), followed by two rich segments [a cysteine-finger region (aa 138 - 184) (also called CR1/C1) and a second cysteine-rich region (CR2) (aa 253 - 264)] and a C-terminal Ser/Thr kinase catalytic domain (aa 354 - 611). Active Raf-1 phosphorylates and activates the MAPK kinases MEK1 and 2, which in turn phosphorylate and activate the MAP kinases ERK1 and 2.