Human/Mouse/Rat Raf-1 Antibody

Monoclonal Mouse IgG_{2A} Clone # 563002

Catalog Number: MAB4540

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	*E. coli*-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 either lyophilized or as a 0.2 μm filtered solution in PBS.

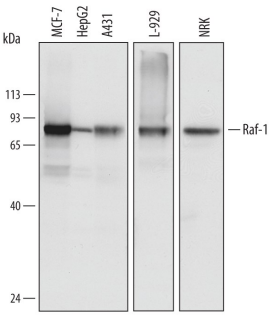
APPLICATIONS

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
Simple Western	10 µg/mL	MCF-7 human breast cancer cell line

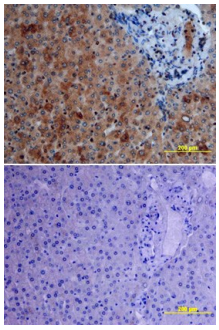
DATA

Western Blot



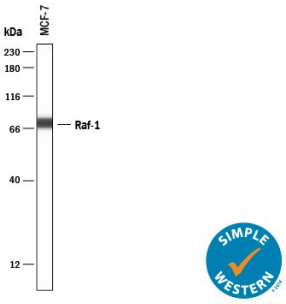
Detection of Human, Mouse, and Rat Raf-1 by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line, HepG2 human hepatocellular carcinoma cell line, A431 human epithelial carcinoma cell line, L-929 mouse fibroblast cell line, and NRK rat normal kidney cell line. PVDF Membrane 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 # 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 # 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.

Simple Western



Detection of Human Raf-1 by Simple Western™. Simple Western lane view shows lysates of MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for Raf-1 at approximately 75 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human/Mouse/Rat Raf-1 Monoclonal Antibody (Catalog # MAB4540) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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Human/Mouse/Rat Raf-1 Antibody

Monoclonal Mouse IgG_{2A} Clone # 563002

Catalog Number: MAB4540

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.** • 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.

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