

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat MDM2.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human MDM2 Asn3-Pro491 Accession # Q00987
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.

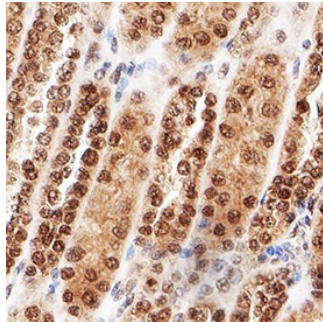
APPLICATIONS

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	3-15 µg/mL	See Below

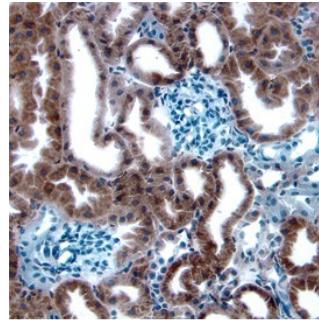
DATA

Immunohistochemistry



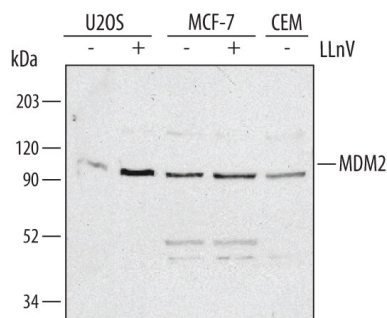
MDM2/HDM2 in Human Kidney. MDM2/HDM2 was detected in immersion fixed paraffin-embedded sections of human kidney using Rabbit Anti-Human/Mouse/Rat MDM2/HDM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1244) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and nuclei for epithelial cells in convoluted tubules. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



MDM2 in Mouse Kidney. MDM2 was detected in perfusion fixed frozen sections of mouse kidney using Human/Mouse/Rat MDM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1244) at 5 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # Catalog # CTS005) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Western Blot



Detection of Human MDM2 by Western Blot. Western blot shows lysates of U2OS human osteosarcoma cell line, MCF-7 human breast cancer cell line, and CEM human T-lymphoblastoid cell line untreated (-) or treated (+) with 5 µM LLnV for 1 hour. PVDF membrane was probed with 1 µg/mL of Human/Mouse/Rat MDM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1244), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # HAF008). A specific band was detected for MDM2 at approximately 95 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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

MDM2 is a key regulator of p53 tumor suppressor protein activity and stability. MDM2 binds to and inhibits the transactivation domain of p53. In addition, MDM2 controls p53 stability by functioning as its E3 ligase in ubiquitination and by shuttling p53 from the nucleus to the cytoplasm for subsequent degradation. The importance of the p53/MDM2 relationship is underscored by the existence of an autoregulatory feedback loop whereby activated p53 transcriptionally up-regulates the expression of its own inhibitor, MDM2.