Human UCH-L1/PGP9.5 Antibody

Monoclonal Mouse IgG_{2A} Clone # 671108

Catalog Number: MAB6007

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human UCH-L1/PGP9.5 in direct ELISAs and Western blots. In direct ELISAs and Western blots, no cross-reactivity with recombinant human UCH-L3 is observed.
Source	Monoclonal Mouse IgG_{2A} Clone # 671108
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human UCH-L1/PGP9.5 Gln2-Ala223 Accession # P09936
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.

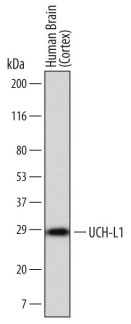
APPLICATIONS

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	2 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Immunoprecipitation	25 µg/mL	Cell lysates spiked with Recombinant Human UCH-L1/PGP9.5 (Catalog # 6007-CY), see our available Western blot detection antibodies
Simple Western	10 µg/mL	See Below
Knockout Validated	UCH-L1/PGP9.5 is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in UCH-L1/PGP9.5 knockout HEK293T cell line.	

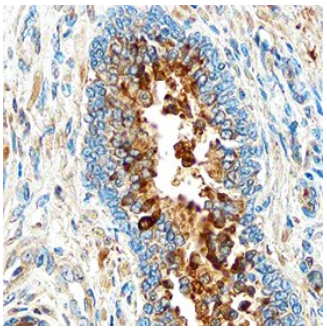
DATA

Western Blot



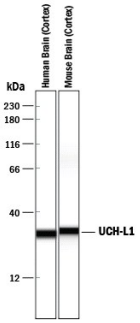
Detection of Human UCH-L1/PGP9.5 by Western Blot. Western blot shows lysates of human brain (cortex) tissue. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human UCH-L1/PGP9.5 Monoclonal Antibody (Catalog # MAB6007) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for UCH-L1/PGP9.5 at approximately 29 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



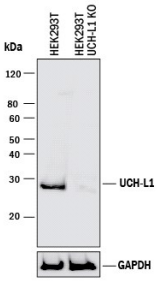
UCH-L1/PGP9.5 in Human Prostate. UCH-L1/PGP9.5 was detected in immersion fixed paraffin-embedded sections of human prostate using Mouse Anti-Human UCH-L1/PGP9.5 Monoclonal Antibody (Catalog # MAB6007) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm of glandular epithelial cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human and Mouse UCH-L1/PGP9.5 by Simple Western™. Simple Western lane view shows lysates of human brain (cortex) tissue and mouse brain (cortex) tissue, loaded at 0.2 mg/mL. A specific band was detected for UCH-L1/PGP9.5 at approximately 32 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human UCH-L1/PGP9.5 Monoclonal Antibody (Catalog # MAB6007). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Knockout Validated



Western Blot Shows Human UCH-L1/PGP9.5 Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and UCH-L1/PGP9.5 knockout HEK293T cell line (KO). PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human UCH-L1/PGP9.5 Monoclonal Antibody (Catalog # MAB6007) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for UCH-L1/PGP9.5 at approximately 28 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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Monoclonal Mouse IgG_{2A} Clone # 671108

Catalog Number: MAB6007

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
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

UCH-L1 (ubiquitin carboxyterminal hydrolase isozyme 1; also PGP9.5) is a 24-27 kDa member of the peptidase C12 family of enzymes. It shows restricted expression, being found in neurons and oocytes. UCH-L1 has dual enzymatic activity. As a monomer, it is a ubiquitin hydrolase that removes ubiquitin from modified proteins; as a homodimer, it acts as a ligase that creates ubiquitin dimers. In neurons, UCH-L1's most important role appears to be that of generating free ubiquitin. Human UCH-L1 is 223 amino acids (aa) in length. It is O-glycosylated, ubiquitinated, and farnesylated; when farnesylated, it becomes associated with cell membranes. Three potential splice forms are reported. One shows a two aa substitution for aa 12-15, a second contains an alternative start site at Met82, and a third shows the same start site coupled with a deletion of aa 138-153. Full-length human UCH-L1 shares 95% aa identity with mouse UCH-L1.

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