Human UCHL1/PARK5 Mouse Monoclonal Antibody (Clone YK-3G8)

Cat# CY-M1040

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
YK-3G8	WB	H	27-28 kDa	Mouse IgG1

Background

The UCHL1, also called neuronal-specific protein gene product 9.5, is a carboxyl-terminal ubiquitin hydrolase regulating ubiquitin dependent signaling pathways, recently suggested as a tumor suppressor. UCHL1 is expressed predominantly in neurons (1), representing 1 to 2% of total soluble brain protein (2), as well as in testis and ovary. In vivo, UCHL1 has been shown to be involved in the regulation of the ubiquitin pool, apoptosis, learning and memory, and its absence in mice because of spontaneous intragenic deletions yields phenotypes with neurological defects (3). Mutations in UCHL1 have been discovered in a German family with Parkinson disease (PD); a point mutation near the active site that changes Ile 93 to Met (I93M), which caused a partial loss of the catalytic activity of this thiol protease. This mutation has been linked to an increased risk of developing an autosomal-dominant form of PD (4).

Based on the abundance in the CNS, UCHL1 has been proposed as a candidate biomarker for brain injury and ischemic strokes. It was demonstrated that UCHL1 was released from injured neurons and flow into the cerebrospinal fluid and eventually into circulating blood (5).

Specificity/Sensitivity: The Human UCHL1 Monoclonal Antibody detects endogenous UCHL1 by western blotting and sandwich ELISA.

Source/Purification: The antibody is produced by immunizing mice with a recombinant full length human UCHL1. IgG is purified by protein A-Sepharose chromatography.

Recommended Antibody Dilutions: Western blotting: 0.5-1 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at –20°C.

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay
Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** *Xenopus* **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:

1. Osaka H, Wang YL, et al. (2003) Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. *Hum Mol Genet* **12:** 1945-58.

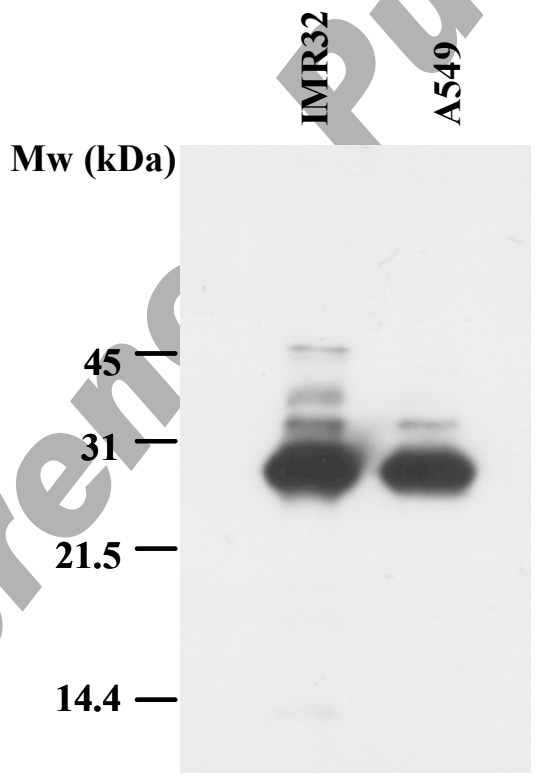
2. Wilkinson, K. D., Lee, K. M., Deshpande, S., Duerksen-Hughes, P., Boss, J. M., Pohl, J. (1989) The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science* **246:** 670-672,

3. Saigoh K, Wang YL, et al. (1999) Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. *Nat Genet* **23:** 47-51.

4. Leroy, E., Boyer, R. et al. (1998) The ubiquitin pathway in Parkinson's disease. Nature **395:** 451-452,.

5. Papa L, Akinyi L et al. (2010) Ubiquitin C-terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. *Crit Care Med.* **38:** 138-44.

Fig.1 Western blot analysis of Human UCHL1 in IMR32 and A549 cell lines



Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

Related Products

*** CircuLex DJ-1/PARK7 ELISA Kit: Cat# CY-9050**

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