Catalog #	Aliquot Size
U522-380H-05	5 µg
U522-380H-10	10 µg

UCHL1, Active

Human full-length recombinant protein expressed in *E. coli* cells

Catalog # U522-380H

Lot # Y901-6

Product Description

Recombinant human UCHL1 was expressed in *E. coli* cells using an N-terminal His tag. The UCHL1 gene accession number is NM_004181.

Gene Aliases

PGP 9.5, UCH-L1, NDGOA, PGP95, SPG79, PARK5.

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

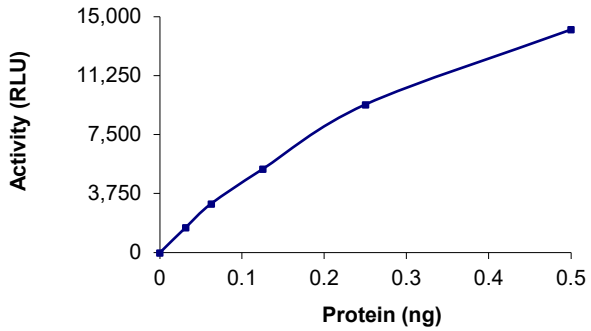
Scientific Background

UCHL1 (ubiquitin carboxyl-terminal esterase L1) is a member of the peptidase C12 family and is a thiol protease that hydrolyzes the C-terminal glycine of ubiquityl esters and amides. It is expressed in the brain and neuroendocrine system and is essential for cytoplasmic protein degradation via recycling free ubiquitin. Mutations in UCHL1 are associated with Parkinson's Disease (PD) due to its ligase and hydrolase activity, both of which are crucial for neuronal health (1). Dysfunction of the enzyme is also associated with Alzheimer's disease as UCHL1 was found to interact with amyloid β precursor protein and regulates the production of amyloid β protein (2).

References

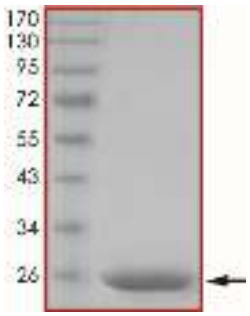
1. Liu, Y. et al: The UCH-L1 Gene Encodes Two Opposing Enzymatic Activities that Affect a-Synuclein Degradation and Parkinson's Disease Susceptibility. Cell 111: 209-218, 2002.
2. Zhang, M. et al: Overexpression of ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) delays Alzheimer's progression in vivo. Scientific Reports, 4:7298, 2014.

Specific Activity



The specific activity of UCHL1 was determined to be **≥401 nmol /min/mg** as per activity assay protocol.

Purity



The purity of UCHL1 was determined to be **>95%** by densitometry, approx. MW **25 kDa**.

UCHL1, Active

Recombinant full-length human protein expressed in *E. coli* cells

Catalog #	U522-380H
Specific Activity	≥401 nmol/min/mg
Lot #	Y901-6
Purity	>95%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Enzyme (Catalog #: U522-380H)

Active UCHL1 (0.1 μg/μl) diluted with DUB Reaction Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active UCHL1 for optimal results).

4X DUB Reaction Buffer (Catalog # D01-09)

Buffer components: 0.2M HEPES (pH7.5), 2mM EDTA, 4mg/ml BSA, add 40mM DTT prior to use. The final concentration of DTT in the assay is 10mM.

Luciferin Detection Reagent (Promega, Cat # V8920)

Luciferin Detection Reagent
Reconstitution Buffer

DUB Substrate I (Catalog # U06-57)

Recombinant human ubiquitin-based proluciferin substrate with a final concentration of 1mg/ml.

Assay Procedure

The UCHL1 deubiquitinase activity is detected using a ubiquitin-based proluciferin substrate that is processed through coupled-enzyme reactions to release a light signal. The light intensity is quantitatively correlated with the enzyme activity.

Step 1. Thaw the Active UCHL1, DUB Substrate I and reaction buffer on ice. Thaw reconstituted Luciferin Detection Reagent at room temperature and keep away from light.

Step 2. Prepare 1X DUB Reaction Buffer containing 10 mM DTT; prepare enzyme and substrate working stock solutions with the buffer.

Step 3. In a half-area solid white 96-well plate, add the following components to bring the reaction volume to 20 μl:

Component 1. 10 μl of 1X DUB Reaction Buffer (replace with test compound solution if performing HTS assay)

Component 2. 5 μl of Active UCHL1 working solution (for HTS assay, pre-incubate for 15 minutes before adding substrate)

Component 3. 5 μl of 2.4 μM substrate working solution

Note 1: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of reaction buffer.

Note 2: A series of aminoluciferin (AML) standard solutions can be included with the enzyme assay in order to determine the specific activity of the enzyme.

Step 4. Briefly centrifuge the plate to ensure reagents are fully mixed and at the bottom of the wells. Incubate the plate at room temperature for 30 minutes.

Step 5. Add 20 μl of Luciferin Detection Reagent to all wells, mix by shaking for 2 minutes. Incubate the plate at room temperature for 30 minutes.

Step 6. Read the plate using the KinaseGlo luminescence protocol on a GloMax plate reader (Promega, Cat# E7031)

Step 7. Using an AML standard curve to determine the concentration of AML produced (μM) and calculate the enzyme specific activity as outlined below.

Enzyme Specific Activity (SA) (nmol/min/mg)

$$= \frac{[AML](\mu M) \times Reaction\ Volume(\mu l)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-3}$$

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: UCHL1, Active **Catalog # U522-380H**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name:	SignalChem Pharmaceuticals Inc.
Street Address:	*110-13120 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixtures.
Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	25%
NaCl	Sodium chloride	7647-14-5	1.75%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	1.34%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	1.02%
Protein		No data available	0.01%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
PMSF; Phenylmethanesulfonyl fluoride	α-Toluenesulphonyl fluoride	329-98-6	0.002%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for sate handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
  Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m³ for mist	British Columbia, Canada
TWA	3 mg/m³ for respirable mist	British Columbia, Canada
TWA	10 mg/m³	Alberta, Canada
TWAEV	10 mg/m³	Ontario, Canada
TWAEV	10 mg/m³	Quebec, Canada
TWA	10 mg/m³	USA

- **Appropriate engineering controls:**
  Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
  Respiratory protection:
  Use appropriate respirator if there is inadequate ventilation by following the government standards.
  Hand protection:
  Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
  Eye/face protection:
  Safety goggles with side-shields approved under appropriate government standards.
  Skin/body protection:
  Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	**Danger of explosion:** Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	**Explosion limits:** Lower: 0.9 Vol %; Upper: 0.0 Vol %.
pH: Not available.	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** 0.1 hPa
Boiling point/Boiling range: 100 °C.	**Density:** Not determined.
Flash point: > 100 °C.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: 400 °C.	**Evaporation rate:** Not determined.
Auto-igniting: Product is not self-igniting.	**Solubility in / Miscibility with Water:** Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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SAFETY DATA SHEET

Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
  Ingestion: May be harmful if swallowed.
  Skin: May be harmful if absorbed through skin. May cause skin irritation.
  Eyes: May cause eye irritation.
- **Signs and Symptoms of Exposure:**
  Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 – Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.