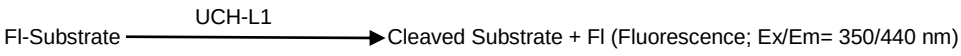
UCH-L1 Deubiquitinase Inhibitor Screening (Fluorometric) Kit

12/15

(Catalog # K484-100; 100 assays; Store at -20°C)

I. Introduction:

Ubiquitin C-terminal Hydrolase 1 (UCH-L1) is a deubiquitinating enzyme (DUB) that is expressed predominantly in neuronal tissue, comprising up to 1-2% of total protein in some brain tissues. UCH-L1 is a cysteine protease and capable of cleaving the isopeptide bond between the carboxyl end of an ubiquitin molecule and a lysine residue on the ubiquitin-modified protein. As ubiquitination is utilized for both localization and degradation of these modified proteins, proper function of deubiquinating enzymes is essential for cell viability and integrity. Dysregulation of UCH-L1 has been implicated in the pathophysiology of neurological disorders like Parkinson's and Alzheimer's diseases, as well as in cancer invasion. A further understanding of these processes can be facilitated by identification of inhibitors with high selectivity. With over 400 DUB enzymes known, this target offers far more selectivity than the proteasome system. BioVision's UCH-L1 Inhibitor Screening Kit utilizes the ability of active UCH-L1 deubiquitinase to cleave a synthetic protein substrate to release the free fluorophore, which can be easily quantified (Ex/Em = 350/440 nm) using a fluorescence microplate reader. Small molecule inhibitors can either reduce or abolish this activity. This inhibitor screening kit thus allows rapid and reliable determination of the inhibitory effects of various compounds on UCH-L1 deubiquitinase and should be used to screen for novel inhibitors.



II. Applications:

- Screening potential inhibitors/ligands of UCH-L1
- Characterizing/studying UCH-L1 inhibitors

III. Kit Contents:

Components	K484-100	Cap Code	Part Number
UCH-L1 Assay Buffer	5 ml	NM	K484-100-1
UCH-L1 Substrate (in DMSO)	25 µl	Red	K484-100-2
UCH-L1 Enzyme	1 vial	Green	K484-100-3
UCH-L1 Inhibitor (LDN-57444)	20 µl	Brown	K484-100-4
96-well Half-Area White Plate	1 Plate	-	K484-100-5

IV. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (ELISA reader)
- DMSO

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **UCH-L1 Assay Buffer:** Ready to use. Warm to room temperature before use. Store at -20°C.
- **UCH-L1 Substrate**: Warm UCH-L1 Substrate to room temperature before use. Light sensitive. Store at -20°C. Use within two months.
- **UCH-L1 Enzyme**: Reconstitute with 220 µl of UCH-L1 Assay Buffer to prepare the stock solution. Aliquot & store at -80°C. Avoid repeated freeze/thaw. Use within two months.
- **UCH-L1 Inhibitor (LDN-57444)**: Inhibitor is supplied to allow user to validate experimental set up and assure that screening will identify inhibitors. There is enough inhibitor for 10 assays.

VI. UCH-L1 Inhibitor Screening Protocol:

1. **UCH-L1 Enzyme Solution Preparation:** Dilute the reconstituted UCH-L1 Enzyme into UCH-L1 Assay Buffer. Prepare enough solution for the number of experiments to be performed. Per well, combine:

UCH-L1 Assay Buffer	36 µl
UCH-L1 Enzyme	2 µl

   Mix Enzyme Solution Preparation well and then add to desired wells in the provided microplate.

2. **Screening compounds, Inhibitor Control & Blank Control preparations:** Dissolve test inhibitors into appropriate solvent; (at least 4% DMSO is tolerated by the UCH-L1 enzyme). For this reason, it is recommended to prepare a 25X stock of test inhibitors; the provided inhibitor is supplied at 25X. For each solvent used, prepare a solvent control. To each well containing enzyme solution, add 2 µl of the supplied 25X inhibitor, 2 µl of the test inhibitor compound, or 2 µl of the solvent control. Incubate 30 minutes at room temperature before substrate addition.
3. **UCH-L1 substrate dilution**: For initial screening, dilute supplied substrate (25 µl) into 1050 µl dH_2O. Alternatively, 5 µl substrate can be diluted into 210 µl dH_2O if not planning to use the whole vial. Do not dilute substrate if not planning on using within 2 hours. Limit light exposure.
4. **Measurement**: After incubation of enzyme with any potential inhibitor compounds and solvent controls, add 10 µl of the diluted UCH-L1 substrate to each well. This will initiate the reaction. Measure the fluorescence in a kinetic mode for 30-60 min at room temperature. (Ex/Em = 350/440 nm). Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).

5. Calculations: Calculate the slope for all test inhibitor samples [S] by dividing the net ΔRFU ($RFU_2 – RFU_1$) values with the time interval ΔT ($T_2 – T_1$).

Note: Compounds that inhibit the UCH-L1 activity completely at the tested concentration will have ΔRFU/Δtime = 0; this indicates 100% relative inhibition.

$$\%\ \textbf{Relative Inhibition} = \frac{\textbf{Slope of EC} - \textbf{Slope of Sample}}{\textbf{Slope of EC}} \times 100$$

$$\%\ \textbf{Relative Activity} = \frac{\Delta\textbf{RFU of S}}{\Delta\textbf{RFU of EC}} \times 100$$

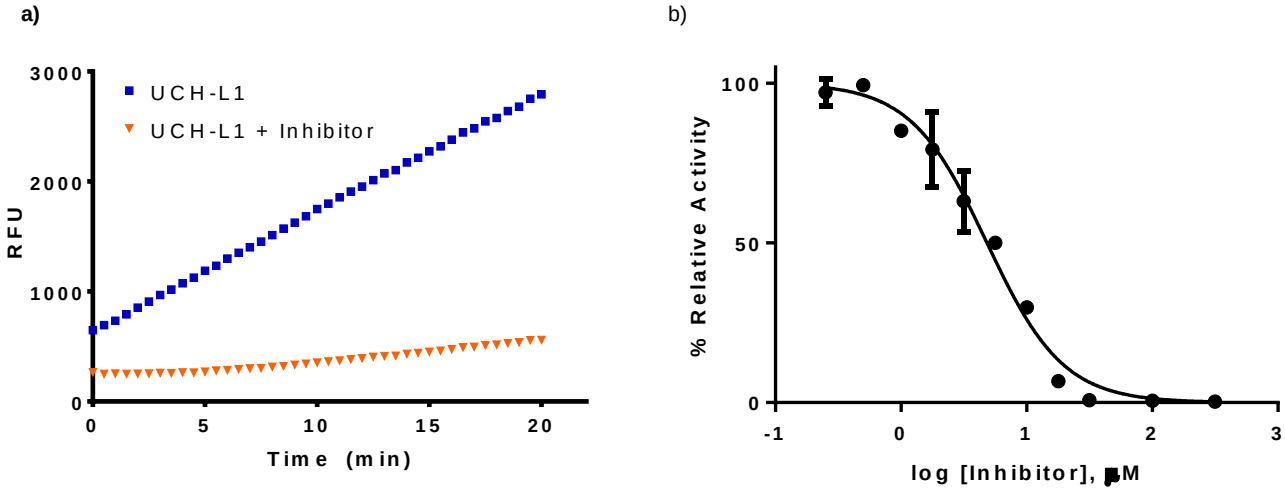


Figure: (a) Sample Inhibition of UCH-L1 enzyme activity by the supplied UCH-L1 Inhibitor**.** (b) Characteristic IC_{50} curve of relative inhibition as a function of treated UCH-L1. The IC_{50} was determined to be 4.828 µM. Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

Human Recombinant UCH-L1 (6306)
Ubiquitin-AMC (4842)
Human Recombinant UCHL3 (6358)
Ubiquitin -Fluorescein-labeled (FLR-Ub) (7552)
NSC-632839 (2570)
TCID (2204)
UCHL1 Polyclonal Antibody (6130)
Ubiquitin-Rhodamine (6411)
Human Recombinant UCHL5 (7847)
LDN-57444 (2016)
PR-619, (2302)
MG-132, (1703)

FOR RESEARCH USE ONLY! Not to be used on humans.