UCH-L3 Deubiquitinase Inhibitor Screening Kit (#BN00705)

(Catalog # BN00705; 100 assays; Store at -20°C)

I. Introduction:

Diseases such as cancer and Alzehimer's are often the result of dysregulation of proteostasis. Protein levels in the cell are regulated by both synthesis and degradation, with some proteins being marked for either localization or proteasomal degradation through the attachment of ubiquitin, a small, 76-amino acid protein. This attachment, termed 'ubiquitination', or 'ubiquitylation' can be reversed by enzymes known as deubiquitinases (DUBs). The human proteome includes as many as four hundred DUBs, and thus these enzymes offer more specific targets for altering cellular equilibrium. UCH-L3, a DUB, is a cysteine protease that has been implicated in prostate cancer, although its regulation and downstream targets are largely unknown. It has been found that UCH-L3 is a novel regulator of epithelial-to-mesenchymal transition in cancer cell metastasis, making it an attractive drug target. Unfortunately, specific inhibitors for this and other DUBs are not readily available. Such compounds would also be a necessity for further illuminating and understanding downstream signaling. Assay Genie's UCH-L3 Inhibitor Screening Kit utilizes the ability of active human UCH-L3 deubiquitinase to cleave a synthetic DUB substrate to release a fluorophore, which can be easily quantified (Ex/Em = 360/460 nm) using a fluorescence microplate reader. This inhibitor screening kit thus allows rapid and reliable determination of the inhibitory effects of various compounds on UCH-L3 deubiquitinase and could be used to screen for novel inhibitors. A nonspecific DUB inhibitor is included in this kit to allow the user to validate the experimental setup.

II. Applications:

- Screening/characterizing potential inhibitors/ligands of UCH-L3
- Analysis of cell signaling pathway

III. Kit Contents:

Components	BN00705	Cap Code	Part Number
UCH-L3 Assay Buffer	10 ml	WM	BN00705-1
UCH-L3 Substrate (in DMSO)	25 µl	Red	BN00705-2
UCH-L3 Enzyme	1 vial	Green	BN00705-3
DUB Inhibitor (50X)	20 µl	Brown	BN00705-4
96-well Half-Area White Plate	1 Plate	-	BN00705-5

IV. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (Fluorescence)
- DMSO

V. Storage Conditions and Reagent Preparation:

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

- **UCH-L3 Assay Buffer:** Ready to use. Warm to room temperature before use. Store at -20°C.
- **UCH-L3 Substrate:** Warm UCH-L3 Substrate to room temperature before use. Light sensitive. Should not be exposed to light for extended periods of time. Store at -20°C. Use within two months.
- **UCH-L3 Enzyme:** Reconstitute with 220 µl of UCH-L3 Assay Buffer to prepare the stock solution. Aliquot & store at -80°C. Avoid repeated freeze/thaw. Use within two months.
- **DUB Inhibitor (50X):** Ready to use. Store at -20°C.

VI. UCH-L3 Inhibitor Screening Protocol:

1. **Screening compounds, Inhibitor Control and Solvent Control preparations:** Dissolve test compounds into appropriate solvent (i.e. DMSO). Add 2 µl test compound or UCH-L3 Assay Buffer into desired wells designated as Sample Compound [S], and Enzyme Control [EC] (no inhibitor) respectively. For DUB Inhibitor Control: dilute necessary amount of supplied inhibitor 1:1 in UCH-L3 Assay Buffer (generating 25X diluted inhibitor control). For the Solvent Control: prepare a 1:1 DMSO:UCH-L3 Assay Buffer mixture. Add 2 µl of 25X diluted inhibitor or 2 µl of the DMSO/Assay Buffer mixture to the wells designated as Inhibitor Control (IC) and Solvent Control (SC) respectively. If any of the test compounds are dissolved in different solvent or DMSO with higher final concentration prepare separate Solvent Control(s) for them.
2. **UCH-L3 Enzyme Solution Preparation:** Prepare enough solution for the number of experiments to be performed. Per well, combine:

UCH-L3 Assay Buffer	36 µl
UCH-L3 Enzyme	2 µl

   Mix Enzyme solution well and then add to desired wells. Incubate 30 minutes at room temperature before substrate addition.
3. **UCH-L3 substrate dilution**: Dilute UCH-L3 substrate 1:44 in UCH-L3 Assay Buffer to generate Working Stock. Prepare enough diluted substrate for the number of reactions to be run. Each well requires 10 µl Working Stock. Add 10 µl Working Substrate Solution to each well containing S, EC, IC and SC.
4. **Measurement**: Measure the fluorescence of each well in a kinetic mode for 30-60 min at room temperature. (Ex/Em = 360/460 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: If the SC slope is significantly different from EC, use SC for the calculations of relative activity.

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

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

a) **b)**

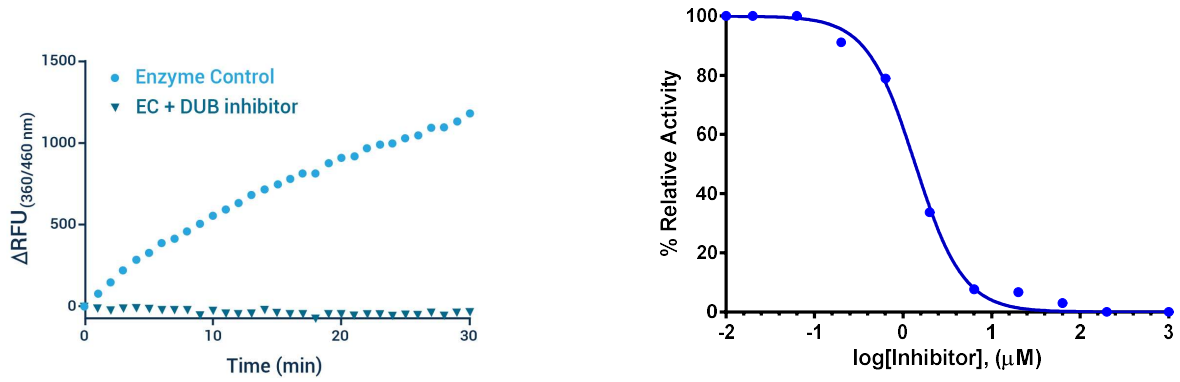

Figure (a) Sample Inhibition of UCH-L3 enzyme activity by the supplied DUB Inhibitor, used at the recommended 1X concentration. Substrate background was subtracted for clarity. (b) Characteristic IC_{50} curve of relative inhibition as a function of treated UCH-L3. The calculated IC_{50} was 1.369 µM.

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