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Deubiquitinating Enzyme Set I

Cat. # J6010

This kit contains 10 deubiquitinating enzymes (DUBs) covering four different DUB families, Each DUB is provided as a 40X stock, and sufficient for 10 X 50 μl (96-well plate) or 25 X 20 μl (384-well plate) reactions. DUBs have different enzymatic activities, each DUB concentration in the kit is tailored to ensure enzymatic activity determination.

Applications:

- DUB activity assay
- DUB profiling assay

Components

	Component	40X Stock Concentration	Quantity
1	40X UCHL1	200 nM	12.5 µl
2	40X UCHL3	20 nM	12.5 µl
3	40X OTUB2	4 μM	12.5 µl
4	40X OTUD2	4 μM	12.5 µl
5	40X 6xHis-AMSH	20 μM	12.5 µl
6	40X GST-USP7	400 nM	12.5 µl
7	40X GST-USP11	600 nM	12.5 µl
8	40X GST-USP15	400 nM	12.5 µl
9	40X 6xHis-USP16	600 nM	12.5 µl
10	40X USP21(195-565)	600 nM	12.5 µl
11	5X Deubiquitination Buffer II	N/A	2 X 1.25 ml

Notes

1. 5X Deubiquitination Buffer II: 250 mM Tris, pH 7.6 at 4 °C, 0.25 M NaCl, 20 mM TCEP, 25% glycerol, and 0.05% Tween-20.
2. The final DUB working concentration and plate reader parameters should be optimized by users.
3. DUBs in the kit are stable in Deubiquitinating Buffer II for at least 2 hours under room temperature.
4. Avoid freeze/thaw cycles for all components. If re-freezing needed, use liquid nitrogen to snap freeze.

Preparation of 1X DUB stock for DUB activity assay

1) Based on your reaction volume (triplicates for each condition is recommended), calculating the amounts of 5X Deubiquitinating Buffer II, distilled water, 40X DUB stock that you will need to make 1X DUB stock.
2) Add appropriate amounts of 5X Deubiquitination Buffer II and ice-cold distilled water in a microfuge tube, gently pipet or tap the tube to mix (do not vortex). Make a master stock if testing multiple enzymes at the same time.
3) For each DUB, mix appropriate amounts of 40X DUB stock and the buffer prepared in Step 2, and gently pipet or tap the tube to mix. Keep the final 1X DUB stock under room temperature.

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Prepare 1X DUB/Compound mixture for profiling assay

1) Final DMSO or other organic solvents should not exceed 1%. Calculate the volume of a testing compound or vehicle (for control reactions) needed to make 1X DUB/compound mixture.
2) Add the appropriate amount of a testing compound stock or vehicle (control) into the bottom of a microfuge tube, add room-temperature distilled water and vortex immediately to dissolve the compound.
3) Add an appropriate amount of 5X Deubiquitination Buffer II and gently pipet or tap the tube to mix (do not vortex).
4) Add an appropriate amount of 40X DUB stock, and gently pipet or tap the tube to mix. Keep the DUB/compound mixture under room temperature at least 15 min to allow the DUB/compound interaction.

Assay DUB activity using Ub-rhodamine 110 as the fluorogenic substrate

1) Ensure plate reader is ready (optimizing plate reader parameters to achieve an excellent signal/noise ratio should be previously performed using one or more DUBs in the kit).
2) To assay DUB activity, add 1 µl 50X (for 50 µl reaction) or 20X (for 20 µl reaction) Ub-rhodamine 110 stock into each well first, then add 1X Deubiquitination Buffer II (with or without vehicle) into control reaction wells, or a 1X DUB or DUB/compound stock prepared above. We recommend the final working concentration of Ub-rhodamine 110 (UBPBio catalog# M3020) at 1 µM.
3) Initiate a kinetic assay immediately.

Representative DUB activity assays

The assays were preformed using the kit components with Ub-rhodamine 110 as the substrate. The rhodamine 110 fluorescence was recorded using a BioTek Synergy HTX plate reader with the excitation and emission filter set at 485/20 and 528/20 nm, respectively. Same reader parameters were used for all DUBs.