

Auto-ubiquitinylation kit

Well-validated test to assess ubiquitin E3 ligase activity

This E3 ligase auto-ubiquitinylation kit enables proteins to be tested for ubiquitin E3 ligase activity through assessment of their ability to under auto-ubiquitinylation. Utilizing the first three steps in the ubiquitin cascade the kit facilitates ubiquitinylation of known or putative E3 ligase enzymes followed by Western blot analysis using the highly sensitive reagents provided or using antibodies to the specific protein of interest (user supplied). A high integrity ubiquitin E3 ligase enzyme is also provided for use as a positive control.

Citations: 17

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Ordering Information

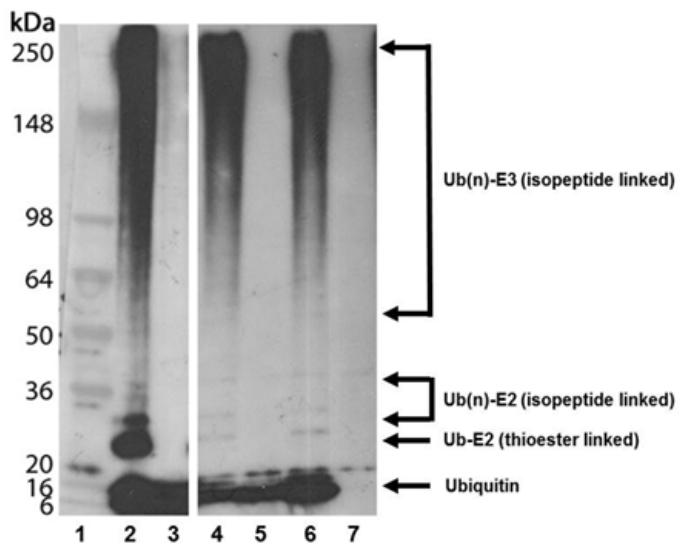
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BML-UW0970-0001	10 tests
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Manuals, SDS & CofA

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- Qualitative assessment of an Ub E3 ligase enzyme's activity through its ability to auto-ubiquitinylate.
- Testing of proteins for auto-ubiquitinylation activity allowing their identification as putative ubiquitin E3 ligases.
- Ubiquitinylation of substrate proteins (user provided) specific to a particular ubiquitin E3 ligase.



Western blot analysis of control Ub E3 ligase Hdm2 RING domain auto-ubiquitylation assays. Auto-ubiquitylation assays set-up and run as described in "Assay protocol". Ubiquitylated E3 ligase species were detected by Western blotting as described in "Analysis by western blotting", using the provided ubiquitin antibody (Prod. No. ADI-SPA-203) at a dilution of 1:1,000. Results demonstrate auto-ubiquitylation of the control Hdm2 RING domain ligase under the given assay conditions. (E2-Ub (thioester linked) is removed in reducing condition. Left – non-reducing; Right – reducing condition.)

Lane 1: Pre-stained Protein Standard. Lane 2: Hdm2-Ub, (+) control, (non-reduced). Lane 3: Hdm2, (-) control, (no Mg-ATP), (non-reduced). Lane 4: Hdm2-Ub, (+) control, (reduced). Lane 5: Hdm2, (-) control, (no Mg-ATP), (reduced). Lane 6: Hdm2-Ub, (+) control, (reduced). Lane 7: Hdm2, (-) control (no ubiquitin), (reduced).

Handling & Storage

Handling Avoid freeze/thaw cycles.

Short Term Storage -80°C

Long Term Storage -80°C

Shipping Dry Ice

Regulatory Status RUO - Research Use Only

Product Details

Application Notes For assessment of protein Ub E3 ligase activity.

Contents

- 20X Ubiquitin Activating Enzyme Solution (E1), 25µl
- 20X Ubiquitin Conjugating Enzyme Solution (E2), 25µl
- 10X Ubiquitin Solution (Ub), 50µl
- 20X Control Ubiquitin Ligase Solution (E3), 25µl
- 20X Mg-ATP Solution, 25µl
- 10X Ub E3 ligase Buffer, 50µl
- Ubiquitin antibody solution, 25µl

Quantity Sufficient for 10 auto-ubiquitinylation assays.

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