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.

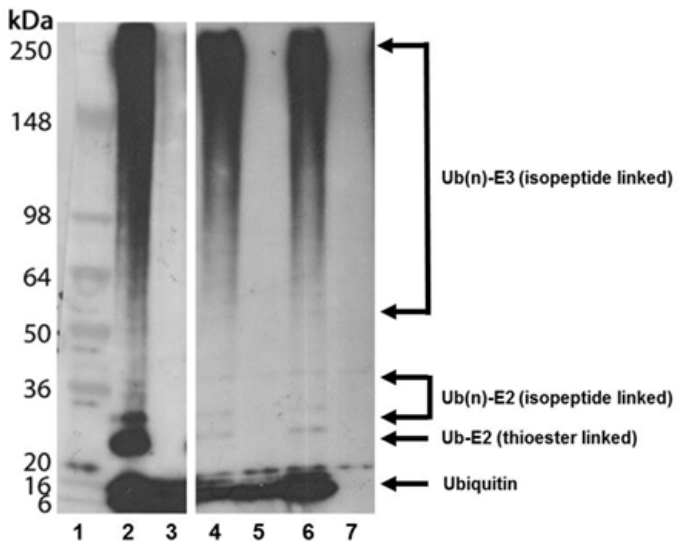
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BML-UW0970-0001	10 tests

Manuals, SDS & CofA View Online »

- 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-ubiquitinylation assays. Auto-ubiquitinylation assays set-up and run as described in “Assay protocol”. Ubiquitinylated 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-ubiquitinylation 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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ENZO LIFE SCIENCES, INC.
Phone: 800.942.0430
info-usa@enzolifesciences.com

European Sales Office
ENZO LIFE SCIENCES (ELS) AG
Phone: +41 61 926 8989
info-eu@enzolifesciences.com

Belgium, The Netherlands & Luxembourg
Phone: +32 3 466 0420
info-be@enzolifesciences.com

France
Phone: +33 472 440 655
info-fr@enzolifesciences.com

Germany
Phone: +49 7621 5500 526
info-de@enzolifesciences.com

UK & Ireland
Phone (UK customers): 0845 601 1488
Phone: +44 1392 825900
info-uk@enzolifesciences.com

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