Ubiquitinylation kit

Versatile tool for generation of ubiquitin-E2 thioesters.

For generation of ubiquitin-E2 thioesters for use in ubiquitinylation experiments. This kit provides the means of generating a range of thioester-linked ubiquitin conjugation enzymes (E2s), utilizing the first two steps in the ubiquitin cascade; for use in the transfer of ubiquitin to E3 ligases; and the subsequent ubiquitinylation of target proteins.Biotinylated ubiquitin provided supports thioester formation and high sensitivity detection of ubiquitin conjugates. Suggested uses: Generation of ubiquitin-E2 thioesters for use in ubiquitinylation experiments, Ubiquitinylation of target proteins in the presence of a dedicated E3 ligase, Activation of ubiquitin for thioester conjugation to novel E2 enzymes, Use of cell lysate or crude fractions/preparations as source of E3 ligases to facilitate ubiquitinylation, Substrate (target) independent in vitro ubiquitinylation reactions.

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Ordering Information

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BML-UW9920-0001	1Kit

Manuals, SDS & CofA

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- Excellent tool for generating a range of thioester-linked ubiquitin-conjugated E2 enzymes
- Biotinylated ubiquitin provided for sensitive detection with streptavidin-linked enzymes
- Kit provides sufficient material for 50 x 50 µl reactions

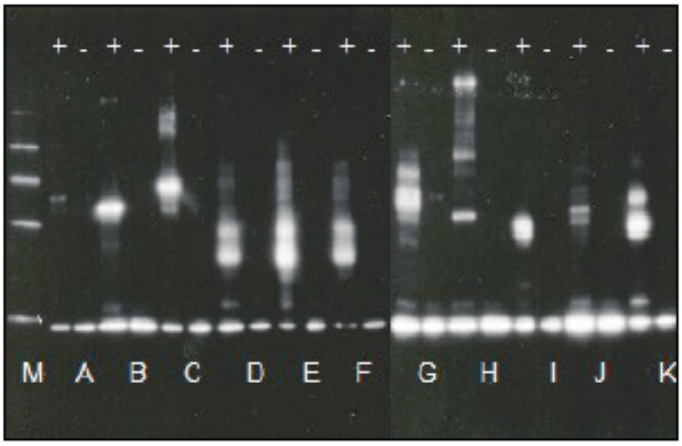


Figure: Western Blot of Thioester Assays (TE +ve/-ve controls) for all E2 conjugating enzymes provided. Procedures as described in “Assay Protocol” section. Biotinylated-ubiquitin-enzyme conjugates were detected by Western Blotting on thioester assays containing **A**: UbcH1 (Prod. No. BML-UW9020), **B**: UbcH2 (Prod. No. BML-UW9025), **C**: UbcH3 (Prod. No. BML-UW8730), **D:** UbcH5a (Prod. No. BML-UW9050), **E**: UbcH5b (Prod. No. BML-UW9060), **F**: UbcH5c (Prod. No. BML-UW9070), **G**: UbcH6 (Prod. No. BML-UW8710), **H**: UbcH7 (Prod. No. BML-UW9080), **I**: UbcH8 (Prod. No. BML-UW9135), **J**: UbcH10 (Prod. No. BML-UW0960), **K** : Ubc13/MMS2 (Prod. No. BML-UW9565) respectively, using Streptavidin-HRP detection system as described in “Analysis by Western Blotting” section. **M**: Biotinylated SDS molecular weight markers (Sigma, SDS-6B) from bottom: 20.1, 29.0, 39.8, 58.1kDa.

Handling & Storage

Use/Stability	All kit components should be stored at -80°C to ensure stability and activity.
Handling	Avoid freeze/thaw cycles.
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application Notes

Uses:

1. Ubiquitinylation of target proteins in presence of dedicated E3 ligase. Panel of E2s provided for generation of E2-Ub thioester conjugates for testing vs. specific E3/target combinations. For example: ubiquitinylation of p53 in the presence of mdm2 (E3) and UbcH5b (E2)10.
2. Activation of Ub for thioester conjugation to novel E2 enzymes (substituted like for like with kit E2s, under directly comparable conditions).
3. Use of cell lysate or crude fractions/preparations as source of E3 ligases to facilitate ubiquitinylation of purified target proteins in the presence of ubiquitinylation kit components.
4. Substrate (target) independent in vitro ubiquitinylation reactions. Determine ubiquitin ligase activity/specificity of proposed E3 enzymes and/or their catalytic domains/fragments11.

Note: Protocols provided for applications 1 and 2. Assay set-up can be readily modified for alternative applications by inclusion, omission or substitution of specific enzyme components.

Contents

20X Ubiquitin Activating Enzyme Solution
Human recombinant E1 (His6-tagged), 125µl provided, Product No. BML-UW9410

10X Ubiquitin Conjugating Enzyme Solutions (E2)
20µl of each E2 provided
UbcH1 (His_6-tagged), Prod. No. BML-UW9020
UbcH2 (His_6-tagged), Prod. No. BML-UW9025
UbcH3 (His_6-tagged), Prod. No. BML-UW8730
UbcH5a (His_6-tagged), Prod. No. BML-UW9050
UbcH5b (His_6-tagged), Prod. No. BML-UW9060
UbcH5c (His_6-tagged), Prod. No. BML-UW9070
UbcH6 (His_6-tagged), Prod. No. BML-UW8710
UbcH7 (His_6-tagged), Prod. No. BML-UW9080
UbcH8 (His_6-tagged), Prod. No. BML-UW9135
UbcH10 (untagged), Prod. No. BML-UW0960
UbcH13/Mms2 (His_6-tagged), Prod. No.BML-UW9565

20X Biotinylated Ubiquitin Solution (Bt-Ub), 125µl provided, Prod. No. BML-UW8705
20X Mg-ATP Solution, 125µl provided, Prod. No. BML-EW9805
2X Non-reducing Gel Loading Buffer, 2.5 ml, Prod. No. BML-KW9880
10X Ubiquitinylation Buffer, 250 µl, Prod. No. BML-KW9885

Quantity

Provides sufficient material for 4 reactions with each included E2 enzyme.

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