UbcH (E2) Enzyme Kit

Catalog Number K-980B

This kit contains reagents for the qualitative analysis of UbcH Enzyme usage.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

Boston Biochem, Inc.
840 Memorial Drive
Cambridge, MA 02139, USA
TEL: (617) 576-2210 FAX: (617) 492-3565
E-MAIL: techsupport@bostonbiochem.com

DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@bio-techne.com

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@bio-techne.com

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

UBE2H (UbcH2) - This E2 enzyme encodes for the human homolog of the yeast Ubc8 gene. UbcH2 can conjugate Ubiquitin to histone H2A in an E3-independent manner *in vitro*. This E2 enzyme is also involved in the Ubiquitination of N-end rule pathway substrates, and may have a role in sepsis-induced muscle protein proteolysis and cancer-induced cachexia.

UBE2R1 (UbcH3) – UBE2R1 plays an essential role in the progression of cells from the G1 to S phase of the cell division cycle. One pathway (requiring Cdc34) initiates DNA replication by degrading a CDK (cyclin-dependent kinase) inhibitor. The second pathway, involves the anaphase-promoting complex (APC) initiates chromosome segregation and exit from mitosis by degrading anaphase inhibitors and mitotic cyclins.

UBE2D1 (UbcH5a) - Human UbcH5a, also known as UBE2D1, is a ubiquitously expressed protein that shares 89% and 88% amino acid sequence identity with the related family members UbcH5b and UbcH5c, respectively. UBE2D1 interacts with a variety of HECT and RING finger Ubiquitin ligases (E3) to mediate the Ubiquitination of specific target proteins. UBE2D1 interacts with E6AP to conjugate Ubiquitin to p53; additional targets of UBE2D1 include c-Fos, RIP1, and HIF-1.

UBE2D2 (UbcH5b) - UbcH5b, also known as UBE2D2, is a widely expressed protein that localizes to both the nucleus and the cytoplasm. Working with the SCF(FbxW2) and HDM2 Ubiquitin ligases, UBE2D2 mediates the Ubiquitination of the transcription factors GCM1 and p53, respectively. UBE2D2 may have a role in the endocytosis and lysosomal degradation of MHC class I molecules, while Ubiquitination of TRIM5 by UBE2D2 has been reported to block HIV reverse transcription.

UBE2D3 (UbcH5c) - Human UbcH5c, also known as UBE2D3, is a member of the yeast Ubc4/5 family of Ubiquitinconjugating enzymes. In combination with various E3 ligases, UBE2D3 mediates the Ubiquitination and subsequent degradation of several regulatory proteins and is implicated in NF-κB-dependent inflammation. UBE2D3 also mediates the Ubiquitination of Histone H2A and PCNA, suggesting that it functions during transcriptional regulation, DNA replication, and DNA damage responses.

UBE2E1 (UbcH6) - Human UbcH6, also known as UBE2E1, is a ubiquitously expressed protein that mediates the selective degradation of shortlived and abnormal proteins. In the nucleus, the UBE2E1 interacts with the Ubiquitin ligase TRIM21 to mediate substrate Ubiquitination. UBE2E1 interacts with the viral E3, ICP0, during HSV-1 infection, and is also implicated in polyglutamine tract disorders such as ataxia.

UBE2E3 (UbcH8) – This enzyme appears to be localized in the nucleus of interphase cells and cellwide in mitotic cells. AutoUbiquitination of ARA54, RNF8, and bacterially-encoded NleG Ubiquitin ligases (E3s) is mediated by UBE2E3. UBE2E3 also functions with NEDD4L to regulate ENaC activity. Physiologically, UBE2E3 is required for the proliferation of retinal pigment epithelial cells via the negative regulation of p27/Kip1 levels, and may also be required for HIV1 replication.

INTRODUCTION *CONTINUED*

UBE2L3 (UbcH7) - UBE2L3 mediates the selective degradation of short-lived and abnormal proteins and is highly homologous to UbcH5. UBE2L3 interacts with the HECT domain of E6AP and the RING domain of c-Cbl, and can mediate the multi-Ubiquitination of many different types of protein substrates.

UBE2C/UbcH10 - UbcH10 is an essential mediator of mitotic destruction events and cell cycle progression. It catalyzes the destruction of cyclins A and B in conjunction with the anaphase-promoting complex. This activity is essential at then end of mitosis for the inactivation of their partner kinase Cdc2 and exit from mitosis into G1 of the next cell cycle.

UBE2N-UBE2V1/ Ubc13-Uev1a - This is a complex of Ubc13 (aka UBE2N, a canonical E2) and Uev1a (UBE2V1, a protein similar to E2's in sequence, but lacking an active site cysteine). This complex functions in the assembly of K63-linked polyUbiquitin chains which have a role in a variety of processes such as DNA repair, endocytosis, polysome stability and signal transduction pathways.

TECHNICAL HINTS

- This kit provides a set of 10 E2 Ubiquitin Conjugating enzymes that are commonly used by E3 Ubiquitin Ligases. However, since there are approximately 40 E2 enzymes found in humans, it is possible that the investigators chosen E3 enzyme might not use any of the E2 enzymes contained in this kit. A suitable positive control reaction should be considered.
- The Ubiquitin E1-E2-E3 conjugation cascade contains two or more transient Ubiquitin-Enzyme thioester conjugates that are sensitive to thiol-based reductants such as beta-mercaptoethanol (βME) or dithiothreitol (DTT). During conjugation reactions, concentrations of these reductants should be kept low (we suggest 0.1-0.5 mM). Higher concentrations of reductant (10 mM) may be used to terminate reactions, particularly when used in conjunction with 10 mM EDTA.
- Complex lysates from eukaryotic cells contain deubiquitinases ("DUBs") that can quickly remove Ubiquitin from a target protein of interest. If the investigator's source of E3 Ligase is a crude lysate or fraction, then the addition of 2-10 µM Ubiquitin-aldehyde, Ubiquitin-Vinyl Sulfone, Ubiquitin-Vinyl Methyl Ester, or Ubiquitin-Propargyl Amide (U-201, U-202, U-203, or U-214) should be considered to help prevent unwanted DUB activity.
- Typical post-reaction analysis of results is accomplished using SDS-PAGE and Western Blots. Primary detection antibody may be chosen against the E3 Ligase (for determining autoubiquitination) or an added test substrate (for determining substrate Ubiquitination). More information is available at techsupport@bostonbiochem.com.

MATERIALS PROVIDED & STORAGE CONDITIONS

Kit contains the following E2 Ubiquitin-conjugating enzymes supplied at 25 µM concentrations.

COMPONENT	VOLUME	STORAGE OF COMPONENTS
UBE2H (UBCH2)	10 µg	Store at -80 °C.* Avoid multiple freeze-thaw cycles.
His_6-UBE2R1 (UbcH3)	10 µg	
UBE2D1 (UbcH5a)	10 µg	
UBE2D2 (UbcH5b)	10 µg	
UBE2D3 (UbcH5c)	10 µg	
His_6-UBE2E1 (UbcH6)	10 µg	
His_6-UBE2E3 (UbcH8)	10 µg	
UBE2L3 (UbcH7)	10 µg	
UBE2C (UbcH10)	10 µg	
His_6-UBE2N/UBE2V1 (Ubc13/Uevla)	10 µg	

* Provided this is within the expiration date of the kit.

OTHER MATERIALS REQUIRED

- **dH_2O:** Sterile
- **Dithiothreitol (DTT):** 1M in dH_2O (Pierce #20290) or similar
- **E1 Ubiquitin Activating Enzyme:** (Boston Biochem, Catalog # E-304, E-305, or E-306), or equivalent
- **Ubiquitin:** (Boston Biochem, Catalog # U-100H), or equivalent
- **E3 Ubiquitin Ligase:** Suitably purified by immunoprecipitation or classical protein purification techniques
- **Buffered ATP Solution:** (Boston Biochem, Catalog # B-71 plus B-20), or equivalent
- **Substrate protein:** Optional

ASSAY PROTOCOL

Reagent Preparation

1. Quickly thaw all protein reagents and buffers by gently and continuously swirling tubes in a lukewarm water bath (≤ 30 °C). Alternatively, tubes may be thawed with a rapid back-and-forth rolling motion between palms of hands. Do not heat tubes for an extended period of time. Do not vortex or shake vigorously.
2. When completely thawed, gently tap tubes to make sure components are well mixed and then briefly spin in a microcentrifuge (5 seconds) to collect contents in bottom of tubes.
3. Immediately ice components. Entire process from steps 1-2 should be accomplished in ≤ 5 minutes.
4. It is **strongly recommended** that each reagent be divided into smaller aliquots to minimize the number of freeze-thaw cycles of kit components. Avoid multiple freeze-thaw cycles to maximize kit performance. Rapidly re-freeze any aliquoted materials in dry ice bath.

Reaction Assembly

1. Prepare reactions on ice in 0.5 or 1.5 mL polypropylene tubes using the following volumes and order of addition:
    - 22 µL dH_2O
    - 5 µL 10X Reaction Buffer. Mix gently following addition.
    - 5 µL 10-50 µM Substrate Protein (Suggested final concentration of 1-5 µM substrate), optional.
    - 5 µL 2-10 µM E3 Ligase Enzyme (suggested final concentration of 0.2-1 µM Ligase).
    - 1 µL 5 µM Ubiquitin E1 Activating Enzyme.
    - 2 µL 25 µM E2 Conjugating Enzyme.
    - 5 µL 100-500 µM Ubiquitin.
2. Volume of water may be adjusted to accommodate other additions or changes in reagent concentrations. Any pre-reaction incubations may be done at this point (e.g. treatment of complex lysates with DUB inhibitors). Addition of Mg^{2+}-ATP in the next step will start the reaction.
3. Add 5 µL of 10X Mg^{2+}-ATP solution (final ATP concentration of 1-10 mM). Mix by gently pipetting up and down 2-3 times. For negative control reactions, omit ATP addition and replace with 5 µL dH_2O.
4. Spin tubes to collect contents and place reactions in 37°C water bath.
5. After 30-90 minutes, terminate reactions with addition of 12 µL 5X Loading Buffer (SDS-PAGE sample buffer) and 3 µL 1M DTT. (An initial time course is recommended to determine the optimal incubation time for efficient substrate conjugation.)
6. Heat reactions to 90 °C for 5 minutes.

Analysis

1. It is left to the investigator to best determine how to analyze results. SDS-PAGE + Western Blot Analysis is commonly used to detect high molecular weight Ubiquitin-E3 Ligase conjugates (autoubiquitination) or Ubiquitin-Substrate conjugates (substrate ubiquitination).

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