

E2-Ubiquitin Conjugation Kit ab139472

6 References 画像数 2

製品の概要

製品名	E2-Ubiquitin Conjugation Kit
サンプルの種類	Purified protein
アッセイタイプ	Enzyme activity
製品の概要	E2-Ubiquitin Conjugation Kit (ab139472) provides the tools necessary for generating a range of thioester-linked ubiquitin-conjugated E2 enzymes for use in ubiquitylation experiments. Biotinylated ubiquitin (provided in the kit) can be detected with streptavidin-linked enzymes via SDS PAGE and western blotting.

This product provides sufficient material for a total of 50 reactions: 4 reactions with each included E2 enzyme.

Uses for this kit include:

1. Ubiquitylation 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: ubiquitylation of p53 in the presence of mdm2 (E3) and UbCH5b (E2).
2. Activation of Ub for thioester conjugation to novel E2 enzymes (substituted like for like with kit E2s, under directly comparable conditions).

特記事項	Ubiquitylation, the covalent attachment of ubiquitin to proteins, is achieved through three enzymatic steps. In an ATP-dependent process, the ubiquitin activating enzyme (E1) catalyzes the formation of a reactive thioester bond with ubiquitin, in the presence of a Mg ²⁺ cofactor, followed by its subsequent transfer to the active site cysteine of a ubiquitin carrier protein (E2). The specificity of ubiquitin ligation arises from the subsequent association of the E2-ubiquitin thioester with a substrate specific ubiquitin-protein isopeptide ligase (E3), which facilitates the formation of the isopeptide linkage between ubiquitin and its target protein.
------	---

製品の特性

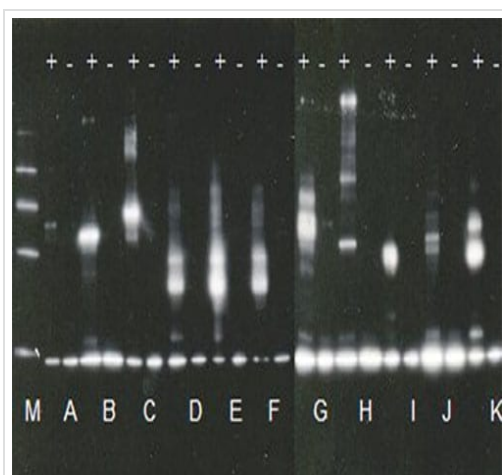
保存方法	Store at -80°C. Please refer to protocols.
------	--

内容	50 tests
10X Ubiquitylation Buffer	1 x 250µl
20x Biotinylated Ubiquitin Solution	1 x 125µl
20X Mg-ATP Solution	1 x 125µl
20X Ubiquitin Activating Enzyme Solution (E1)	1 x 125µl
2X Non-reducing Gel Loading Buffer	2 x 1.25ml
UbcH1 (Human, Recombinant)	1 x 20µl
UbcH10 (Human, Recombinant)	1 x 20µl
UbcH13/Mms2 (Human, Recombinant)	1 x 20µl
UbcH2 (Human, Recombinant)	1 x 20µl
UbcH3 (Human, Recombinant)	1 x 20µl
UbcH5a (Human, Recombinant)	1 x 20µl
UbcH5b (Human, Recombinant)	1 x 20µl
UbcH5c (Human, Recombinant)	1 x 20µl
UbcH6 (Human, Recombinant)	1 x 20µl
UbcH7 (Human, Recombinant)	1 x 20µl
UbcH8 (Human, Recombinant)	1 x 20µl

関連性

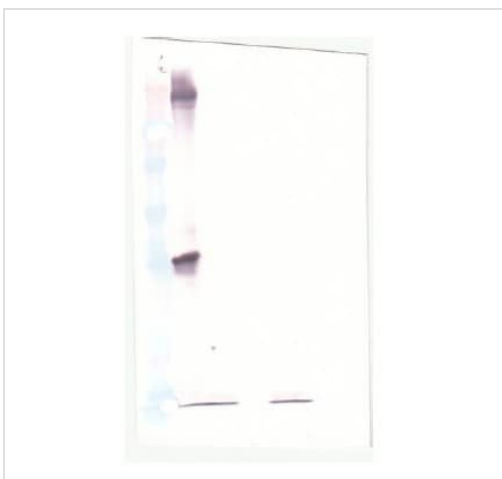
Ubiquitin-conjugating enzymes, also known as E2 enzymes and more rarely as ubiquitin-carrier enzymes, perform the second step in the ubiquitination reaction that targets a protein for degradation via the proteasome. The ubiquitination process covalently attaches ubiquitin, a short protein of 76 amino acids, to a lysine residue on the target protein. Once a protein has been tagged with one ubiquitin molecule, additional rounds of ubiquitination form a polyubiquitin chain that is recognized by the proteasome's 19S regulatory particle, triggering the ATP-dependent unfolding of the target protein that allows passage into the proteasome's 20S core particle, where proteases degrade the target into short peptide fragments for recycling by the cell.

画像



Western Blot of Thioester Assays (TE +ve/-ve controls) for all E2 conjugating enzymes provided

Biotinylated-ubiquitin-enzyme conjugates were detected by Western Blotting on thioester assays containing UbchH1 (A), UbchH2 (B), UbchH3 (C), UbchH5a (D), UbchH5b (E), UbchH5c (F), UbchH6 (G), UbchH7 (H), UbchH8 (I), UbchH10 (J), Ubch13/MMS2 (K) respectively, using Streptavidin-HRP detection system. M: Biotinylated SDS molecular weight marker, from bottom: 20.1, 29.0, 39.8, 58.1kDa.



Western blot analysis of Thioester assays (TE (+)ve/(-)ve control)

Western blot analysis of Thioester assays (TE (+)ve/(-)ve control) for E2 conjugating enzyme UbchH2. Procedure as described in the assay set up section (without DTT), and ubiquitin conjugate detected by streptavidin AP. The absence of conjugate in TE (-)ve control control demonstrate that formation of TE is ATP dependent (required for E1 activation).

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish

- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.co.jp/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors