

E3 Ligase Auto-Ubiquitylation Assay Kit ab139469

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Overview

Product name	E3 Ligase Auto-Ubiquitylation Assay Kit
Sample type	Purified protein
Assay type	Enzyme activity
Product overview	<p>Abcam E3 Ligase Auto-Ubiquitylation Assay Kit (ab139469) enables proteins to be tested for ubiquitin E3 ligase activity through assessment of their ability to undergo auto-ubiquitylation (also known as ubiquitination). Utilizing the first three steps in the ubiquitin cascade the kit facilitates ubiquitylation 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). Hdm2 ubiquitin E3 ligase enzyme is also provided for use as a positive control.</p>

The Kit provides sufficient material for approximately 10 auto-ubiquitylation assays.

Suggested uses for this kit include:

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

Notes	<p>The covalent attachment of ubiquitin to proteins (ubiquitylation or ubiquitination) plays a fundamental role in the regulation of cellular function through biological events involving cell cycle, differentiation, immune responses, DNA repair, chromatin structure, and apoptosis.</p> <p>Ubiquitylation 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, followed by its subsequent transfer to the active site cysteine of a ubiquitin carrier protein (E2). The selectivity of the ubiquitin cascade for a particular substrate protein relies on the interaction between the E2 conjugating enzyme (of which a cell contains relatively few) and a ubiquitin-protein ligase (E3), of which over 600 have been identified to date.</p> <p>The E3s are a large, diverse group of proteins, characterized by one of several defining motifs. These include a HECT (homologous to E6-associated protein C-terminus), RING (really interesting new gene) or U-box (a modified RING motif without the full complement of Zn²⁺-binding</p>
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ligands) domain. Whereas HECT E3s have a direct role in catalysis during ubiquitinylation, RING and U-box E3s facilitate protein ubiquitinylation. These latter two E3 types act as adaptor-like molecules. They bring an E2 and a substrate into sufficiently close proximity to promote the substrate's ubiquitinylation. Although many RING-type E3s, such as MDM2 and c-Cbl, can apparently act alone, others are found as components of much larger multi-protein complexes, such as the anaphase-promoting complex.

Taken together, these multifaceted properties and interactions enable E3s to provide a powerful, and specific, mechanism for protein clearance within all cells of eukaryotic organisms utilising the ubiquitin-proteasome system. The importance of E3s is highlighted by the number of normal cellular processes they regulate, and the number of diseases associated with their loss of function or inappropriate targeting.

E3 ligases also undergo auto-ubiquitinylation, through modification of specific lysine residues within an individual ligase, providing a mechanism thought to be responsible for the regulation of the E3 enzyme itself.

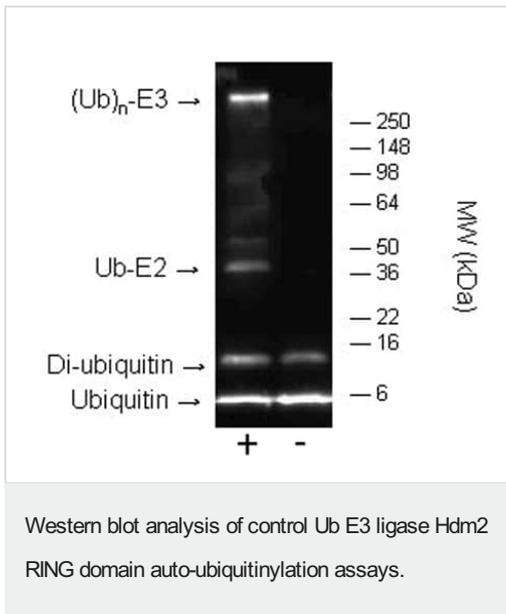
Properties

Storage instructions

Please refer to protocols.

Components	10 tests
10X Ub E3 Ligase Buffer	1 x 50µl
10X Ubiquitin	1 x 50µl
20X Mg-ATP Solution	1 x 25µl
20X Ubiquitin Activating Enzyme Solution (E1)	1 x 25µl
20X Hdm2 (Human, Recombinant)	1 x 25µl
20X Ubch5a (Human, Recombinant)	1 x 25µl
Ubiquitin Antibody Solution	1 x 25µl

Images



Auto-ubiquitylation assays set-up and run as described in Assay protocol. Ubiquitylated E3 ligase species were detected by Western blotting as described in Western Blot Analysis, using the provided ubiquitin antibody at a dilution of 1/1000 dilution.

(+) reaction with Mg-ATP

(-) reaction without Mg-ATP

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