

# Ubiquitin activating kit

For activation of ubiquitin for use in ubiquitinylation experiments. The kit provides the means of generating thioester linked, activated ubiquitin-E1 conjugates, utilizing the first step in the ubiquitin cascade, for investigation of ubiquitin activation, subsequent ubiquitin transfer to/interaction with E2 conjugating enzymes, and their use in the ubiquitinylation of E3 ligases and target substrate proteins. Ubiquitin antibody provided allows high sensitivity detection of ubiquitin and ubiquitin-conjugates.

The covalent attachment of ubiquitin to proteins (ubiquitinylation) and their subsequent proteasomal degradation 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.

Ubiquitinylation 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 an  $Mg^{2+}$  cofactor, followed by its subsequent transfer to the active site cysteine of an 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

Citations: 5

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## Ordering Information

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BML-UW0400A-0001

20 tests

## Manuals, SDS & CofA

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- For use with wild type or mutant E2 enzymes in E1 initiated/mediated reactions
- Contains highly sensitive ubiquitin antibody for detection of ubiquitin and ubiquitin conjugates

## Handling & Storage

Long Term Storage      -80°C

Shipping      Dry Ice

**Regulatory Status** RUO - Research Use Only

## Product Details

### Application Notes

### Uses:

1. Activation of Ubiquitin for conjugation to wild type or mutant E2 enzymes (user supplied) via thioester bond formation.
2. Use of Ub-E2 conjugates produced in the subsequent ubiquitin modification of specific target proteins in presence of a dedicated ubiquitin E3 ligase.
3. Investigation of ubiquitin activation by E1 activating enzyme.
4. Substitution of the wild type ubiquitin provided with ubiquitin mutants or derivatives (*e.g.* biotinylated-ubiquitin, Prod. No. BML-UW8705) allowing their activation and subsequent utility in the ubiquitin cascade.

### Contents

20X Ubiquitin Activating Enzyme Solution (E1)  
20X Ubiquitin Solution (Ub)  
Ubiquitin Antibody Solution  
20X Mg-ATP Solution  
2X Non-reducing Gel Loading Buffer  
10X Ubiquitylation Buffer

### Quantity

20 tests

### UniProt ID

P0CG47 (UBB), P0CG48 (UBC), P62979 (RPS27A),  
P62987 (UBA52)

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