

# Misfolded Protein Ubiquitination Kit

(Cat. # J5110)

\* This kit is enough for setting up 20 x 20  $\mu$ l reactions.

## Description

This kit is designated for monitoring CHIP-mediated ubiquitination of user-supplied misfolded proteins. CHIP is an E3 ubiquitin ligase that directly or via Hsp70 interacts with misfolded proteins and promotes their polyubiquitination, which targets misfolded proteins for proteasomal degradation. HSP70 is not required for CHIP-mediated in vitro ubiquitination.

## Components

- |                                     |             |
|-------------------------------------|-------------|
| • 10X Human Ubiquitin (500 $\mu$ M) | 40 $\mu$ l  |
| • 20X UBE1 (E1, 2 $\mu$ M))         | 20 $\mu$ l  |
| • 10X 6xHis-UbE2D3 (E2, 20 $\mu$ M) | 40 $\mu$ l  |
| • 20X 6XHis-CHIP (E3, 40 $\mu$ M))  | 20 $\mu$ l  |
| • 5X Ubiquitination Buffer          | 150 $\mu$ l |
| • 10X ATP (40 mM)                   | 60 $\mu$ l  |

### 5X Ubiquitination Buffer

100 mM Tris, pH7.2, 250 mM NaCl, 25 mM MgCl<sub>2</sub>, 5 mM 2-mecaptoenthanol, 50% glycerol

## Setting up ubiquitination assay

A 20  $\mu$ l CHIP-mediated ubiquitination reaction contains 100 nM E1, 2  $\mu$ M UbE2D3, 2  $\mu$ M CHIP, 50  $\mu$ M Ub, 1-4  $\mu$ M substrate protein (not provided), and 4 mM ATP in 1X Ubiquitination Buffer. Incubate the reaction mixtures in a 37°C water bath for 1-2 hr and stop the reaction by adding 5  $\mu$ l 5x SDS Sample Buffer (not supplied). Ubiquitination of a substrate protein can be detected by immunoblotting using an antibody recognizing the substrate protein. A reaction without E1 or ATP can be used as a negative control.

