

## Ubiquitin Linkage Screening Kit III (Cat. # J3220)

\*The supplied UBE1 is sufficient for setting up 50 x 20  $\mu$ l reactions. Each Ub mutant is sufficient for setting up 10 X 20  $\mu$ l reactions.

### **Description**

Ubiquitin Linkage Screening Kit III is designed for two purposes: 1) to identify the ubiquitin lysine residue that is used to link the polyubiquitin chain conjugated on a protein substrate; 2) to determine whether a proteinsubstrate is polyubiquitinated or conjugated with multiple monoubiquitin.

A typical 20  $\mu$ l reaction contains 100 nM UBE1, 2  $\mu$ M E2 (not supplied), 50  $\mu$ M Ub or Ub mutants, 4 mM ATP, 2  $\mu$ M E3 (not supplied) and 2  $\mu$ M substrate (no supplied) in the ubiquitination buffer.

### **Components**

- |  |             |
|--|-------------|
| • 20X UBE1 (2 $\mu$ M)                 | 50 $\mu$ l  |
| • 10X 6xHis-Ub (500 $\mu$ M)           | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K0) (500 $\mu$ M)       | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K6 only) (500 $\mu$ M)  | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K11 only) (500 $\mu$ M) | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K27 only) (500 $\mu$ M) | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K29 only) (500 $\mu$ M) | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K33 only) (500 $\mu$ M) | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K48 only) (500 $\mu$ M) | 20 $\mu$ l  |
| • 10X 6xHis-Ub(K63 only) (500 $\mu$ M) | 20 $\mu$ l  |
| • 10X ATP (40 mM)                      | 125 $\mu$ l |
| • 10X Ubiquitination Buffer            | 1 ml        |

### **Note**

1. 10X Ubiquitination Buffer: 400 mM Tris, pH 7.1 at 37°C, 400 mM NaCl, 10 mM  $\beta$ ME, 50 mM  $MgCl_2$

