

Customized E2 Screening Kit (Cat. # J1200)

Description

The E2 Screening Kit is developed for identification of the E2 Ub-conjugating enzyme(s) for a specific E3 Ub ligase and/or a protein substrate.

Components

- 20X UBE1 (2 μ M) 50 μ l
- 10X E2 Ub conjugating enzymes
(pick 10 E2s from the list below, 20 μ M) 20 μ l each
- 10X Human Ubiquitin (500 μ M) 100 μ l
- 10X Ubiquitination Buffer 500 μ l
- 40 mM ATP 125 μ l

Note

	E2 Name		E2 Name
1	6XHis-Ube2A (Rad6A)	16	6XHis-Ube2J1 ₍₁₋₂₈₂₎
2	6XHis-Ube2B	17	GST-Ube2K (E2-25K)
3	6XHis-Ube2C(UbcH10)	18	6XHis-Ube2L3
4	6XHis-Ube2D1 (UbcH5A)	19	6XHis-Ube2L6
5	6XHis-Ube2D2 (UbcH5B)	20	6XHis-Ube2M (UbcH12)
6	6XHis-Ube2D3 (UbcH5C)	21	6XHis-UbeN (Ubc13/Uev1a)
7	6XHis-Ube2D4 (UbcH5D)	22	6XHis-Ube2Q2
8	6XHis-Ube2E1 (UbcH6)	23	6XHis-Ube2R1 (Cdc34)
9	6XHis-Ube2E2 (UbcH8)	24	6XHis-Ube2R2
10	6XHis-Ube2E3 (UbcH9)	25	6XHis-Ube2S
11	6XHis-Ube2F	26	6XHis-Ube2T
12	6XHis-Ube2G1	27	6xHis-Ubc13/Ube2V2
13	6XHis-Ube2G2	28	6XHis-Ube2W
14	6XHis-Ube2H (UbcH2)	29	6XHis-Ube2Z
15	6XHis-Ube2I		

1. We recommend to set up 20 μ l ubiquitination reaction containing 100 nM E1, 2 μ M E2, 2 μ M E3, 2 μ M substrate, 50 μ M Ub, 2 mM ATP, 1 μ l glycerol and 2 μ l 10X Ubiquitination Buffer. Substrate ubiquitination can be assayed by immunoblotting. The provided E1 and Ub are enough for setting up 50 reactions; each E2 enzyme is enough for 10 reactions.
2. 10 X Ubiquitination Buffer: 200 mM Tris, pH 7.6 at 4 $^{\circ}$ C, 500 mM NaCl, 10 mM β ME and 50 mM MgCl₂.
3. Store all components at -80 $^{\circ}$ C upon receiving. Avoid multiple freeze-thaws.

