

E2 Screening Kit (Cat# J1100)

Description

The E2 Screening Kit (Cat# J1100) contains ubiquitin (Ub)-activating enzyme (Ube1), Ub and 29 Ub-conjugating enzymes (E2s). It can be used for 1) identification of the E2 enzyme paired for a specific Ub ligase (E3), and 2) assessing/profiling E2 enzymatic activity by monitoring the E2-Ub thioester conjugate formation (Ub charging assay) under a non-reducing condition.

A few E2s are specific for Ub-like proteins, including SUMO, Nedd8 and ISG15. Please use the E2 Screening Kit II (Cat# J1300) that contains additional SUMO-activating E1, Nedd8-activating E1, SUMO2 and Nedd8 proteins.

List of E2s and their reactivity

	E2 Name	Reactivity
1	6XHis-UbE2A	Ub
2	6XHis-UbE2B	Ub
3	6XHis-UbE2C	Ub
4	6XHis-UbE2D1	Ub
5	6XHis-UbE2D2	Ub
6	6XHis-UbE2D3	Ub
7	6XHis-UbE2D4	Ub
8	6XHis-UbE2E1	Ub
9	6XHis-UbE2E2	Ub
10	6XHis-UbE2E3	Ub
11	6XHis-UbE2F	Nedd8
12	6XHis-UbE2G1	Ub
13	6XHis-UbE2G2	Ub
14	6XHis-UbE2H	Ub
15	6XHis-UbE2I	SUMO

	E2 Name	Reactivity
16	6XHis-UbE2J1 ₍₁₋₂₈₂₎	Ub
17	6xHis-UbE2K	Ub
18	6XHis-UbE2L3	Ub
19	6XHis-UbE2L6	ISG15, Ub
20	6XHis-UbE2M	Nedd8
21	6XHis-UbE2N	Ub
22	6XHis-UbE2Q2	Ub
23	6XHis-UbE2R1	Ub
24	6XHis-UbE2R2	Ub
25	6XHis-UbE2S	Ub
26	6XHis-UbE2T	Ub
27	6xHis-Ubc13/UbE2V2	Ub
28	6XHis-UbE2W	Ub
29	6XHis-UbE2Z	Ub, FAT10
	UbE2Z works specifically with UBA6 E1.	

Components

- 20X UBE1 (2 μ M) 100 μ l
- 10X E2 Ub-conjugating enzymes, 29 E2s
(Listed in the table above, 20 μ M each) 20 μ l each
- 10X Human Ubiquitin (500 μ M) 200 μ l
- 10X Ubiquitination Buffer 1ml
- 20X ATP solution (40 mM) 250 μ l

Notes

1. Reaction conditions should be optimized for specific assays. We recommend an initial testing condition as the following for detecting protein ubiquitination: a 20 μ l ubiquitination reaction contains 100nM E1, 2 μ M E2, 2 μ M E3, 2 μ M substrate protein, 50 μ M Ub, 2mM ATP, 10% glycerol and 1X Ubiquitination Buffer (see table below and follow the addition order to mix components, tap the tube to mix well once water, ubiquitination buffer and glycerol are added). A negative control reaction can be included by omitting ATP in reaction mixtures. Incubate all reactions in a 37 °C water bath for 2 hours. Stop reactions by mixing with reducing SDS sample buffer. Protein ubiquitination can be detected by immunoblotting or Coomassie staining after SDS-PAGE separation. A similar reaction can be used to detect E3 autoubiquitination without a protein substrate.

Components	Reaction Concentration	Volume to be added for a 20 μ l reaction
Purified water	Not provided	6 μ l
10X Ubiquitination Buffer	1X	2 μ l
Glycerol	10% (not provided)	2 μ l
10X Human Ub	50 μ M	2 μ l
10X Protein Substrate	2 μ M (not provided)	2 μ l
10X E2 Ub-conjug. Enzyme	2 μ M	2 μ l
10X E3 Ub Ligase	2 μ M (not provided)	2 μ l
20X UbE1	100nM	1 μ l
20X ATP	2mM	1 μ l

2. For monitoring the thioester conjugate formation (Ub charging) between an E2 and Ub, a 20 μ l Ub charging reaction contains 100nM E1, 2 μ M E2, 50 μ M Ub, 2mM ATP, 10% glycerol and 1X Ubiquitination Buffer. Non-reducing sample buffer should be used to stop the reaction and preserve the thioester bond-linked conjugates. Samples can be heated at 90 °C for 5 minutes. E2-Ub conjugates can be detected by immunoblotting or Coomassie staining after SDS-PAGE separation.

We have a sensitive TR-FRET based E2-Ub charging assay kit (Cat# T5000).

3. 10X Ubiquitination Buffer: 200mM Tris, pH7.6 at 4°C, 500mM NaCl, 10mM β ME and 50mM MgCl₂. This buffer supports Ub charging reaction.
4. Store all components at -80°C upon receiving. Avoid multiple freeze-thaws.