E2 Screening Kit II (Cat# J1300)

Description

The E2 Screening Kit II (Cat# J1300) contains ubiquitin (Ub)-activating enzyme (UbE1), SUMO-activating enzyme (6xHis-SAE1/SAE2), Nedd8-activating enzyme (APPBP1/6xHis-UBA3), Ub, SUMO2, Nedd8, 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) profiling E2 activity by monitoring the E2-Ub thioester conjugate formation under a non-reducing condition.

Comparing to the E2 Screening Kit (Cat# J1100), this kit contains additional SUMO or Nedd8-activating E1, SUMO2 and Nedd8, which allows to assess SUMOylation and neddylation.

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	GST-UbE2K	Ub
18	6XHis-UbE2L3	Ub
19	6XHis-UbE2L6	Ub, ISG15
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 (2μM) 100μl
- 20X 6xHis-SAE1/SAE2 (2μM, SUMO E1) 25μl
- 20X APPBP1/6xHis-UBA3) (2μM, Nedd8 E1) 25μl
- 10X E2 Ub-conjugating enzymes (29 E2s, see table above, 20μM each) 20μl each
- 10X Human Ubiquitin (500μM) 200μl
- 10X SUMO2 (500μM) 25μl
- 10X 6XHis-Nedd8 (150 μM) 50μl
- 10X Ubiquitination Buffer 1ml
- 20X ATP (40mM) 250μl

Notes

1. Reaction conditions should be optimized for specific assays. We recommend an initial testing condition as the following for detecting protein ubiquitination: a 20µl ubiquitination reaction contains 100nM E1, 2µM E2, 2µM E3, 2µM substrate protein, 50µM Ub/50µM SUMO2/15µM Nedd8, 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 ^{0}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 20µl reaction**
Purified water	Not provided	6µl
10X Ubiquitination Buffer	1X	2µl
Glycerol	10% (not provided)	2µl
10X Human Ub, SUMO2 or Nedd8	50µM for Ub/SUMO2 15µM for Nedd8	2µl
10X Protein Substrate	2µM (not provided)	2µl
10X E2 Ub-conjug. Enzyme	2µM	2µl
10X E3 Ub Ligase	2µM (not provided)	2µl
20X UbE1	100nM	1µl
20X ATP	2mM	1µl

2. For monitoring the thioester conjugate formation between an E2 and Ub/SUMO2/Nedd8, a 20 µl Ub charging reaction contains 100nM E1, 2µM E2, 50µM Ub/SUMO2 or 15µM Nedd8, 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 ^{0}C for 5 minutes. E2-Ub conjugates can be detected by immunoblotting or Coomassie staining after SDS-PAGE separation.
3. 10X Ubiquitination Buffer: 200mM Tris, pH7.6 at 4°C, 500mM NaCl, 10mM βME and 50mM $MgCl_2$. This buffer is suitable for ubiquitination, SUMOylation and neddylation reactions.
4. Store all components at -80°C upon receiving. Avoid multiple freeze-thaws.