Ubiquitin E2 Screening Kit

Cat# J1100

Description

The Ubiquitin 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) for a protein substrate, and 2) assessing/profiling E2 activity by monitoring the E2-Ub thioester conjugate formation under non-reducing condition.

A few E2s are specific for other Ub-like proteins, including SUMO, Nedd8 and ISG15. If you need to assess SUMO and Nedd8 reactivity, please use the E2 Screening Kit II (Cat# J1300) that contains additional SUMO or Nedd8-activating E1, as well as 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	GST-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 in the kit

- 20X UBE1 (2μM) 100μl
- 10X E2 Ub-conjugating enzymes
  (29 E2s, see table above, 20μM each) 20μl each

- 10X Human Ubiquitin (500μM) 200μl
- 10X Ubiquitination Buffer 1ml
- 40mM ATP 250μl

Note

1. Reaction conditions should be optimized for specific assays. We recommend an initial testing condition as the following: a 20 μl ubiquitination reaction contains 100nM E1, 2μM E2, 2μM E3, 2μM substrate, 50μM Ub, 2mM ATP, 1μl glycerol and 2μl 10X Ubiquitination Buffer. Substrate ubiquitination can be assayed by immunoblotting. According to this setup, the provided UbE1 and Ub are sufficient for 100 reactions; each E2 enzyme is enough for 10 reactions.
2. For monitoring the thioester conjugate formation between E2 and Ub to assess E2 activity, run non-reducing SDS-PAGE to preserve the thioester bond-linked conjugates. Samples can be heated at 90 ^{0}C for 5min to denature proteins prior to gel running.
3. 10X Ubiquitination Buffer: 200mM Tris, pH7.6 at 4°C, 500mM NaCl, 10mM βME and 50mM $MgCl_2$.
4. Store all components at -80°C upon receiving. Avoid multiple freeze-thaws.