Lot #XXXXX

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MATERIAL DATA SHEET

SUMO Conjugation Reaction Buffer Kit
Cat. # SK-15

SUMO conjugation reaction buffer kit contains the optimal buffer formulations for use in assays for the conjugation of the ubiquitin-like modifiers, SUMO-1 **(UL-712)**, SUMO-2 **(UL-752)**, and SUMO-3 **(UL-762)**, to protein substrates *in vitro*. These SUMO proteins are conjugated to a variety of proteins in the presence of Ubc9 **(E2-645)** and SAE1/SAE2 (human) **(E-315)**. This kit can supplement the SUMO conjugation kits **(K-712, K-715, K-720)**.

Product Information

Stocks:
1. 1ml 10X SUMO Reaction Buffer
2. 100µl 10X Mg-ATP
3. 1ml 50X E1 Stop Buffer
4. 1ml 5X Non-reducing gel loading buffer

Use & Storage

Storage: Store at -20°C. Avoid multiple freeze/thaw cycles.

Background

The ubiquitin-like SUMO-1 is conjugated to a variety of proteins in the presence of Ubc9 and the SUMO E1 activating enzyme (SAE1/SAE2 in human, or Aos1/Uba2p in yeast). The heterodimeric SAE1/SAE2 complex (38 and 70 kDa respectively) uses ATP to adenylate the C-terminal glycine residue of SUMO-1, forming a high-energy thioester bond with the SAE2 subunit. The second step is the trans-esterification reaction whereby the activated SUMO-1 is transferred to Cys^{93} of Ubc9. Ubc9 is a member of the E2 family and is homologous to ubiquitin-conjugating enzymes, but is specific for the conjugation of SUMO to a variety of target proteins. This E2 is unusual in that it interacts directly with protein substrates that are modified by sumolyation, and may play a role in substrate recognition. Sumoylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IκBα, GLUT1, GLUT4). SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation and transcriptional regulation, apoptosis and protein function and stability.

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Recommended Assay Protocol

Protocol (20 µL reaction):

1. Add 2 µL reaction buffer (1X final) to appropriate enzyme and substrate mix.
2. Bring the volume to a total of 18 µL with dH2O if needed.
3. Initiate reaction with the addition of 2 µL of Mg-ATP solution (1 mM final).
4. Incubate the reaction at desired temperature and time.
5. Dilute 50X stop buffer in dH2O to 10X and stop reaction by adding 2 µL of 10X stop buffer.
6. Add 4 µL 5X loading buffer for SDS-PAGE/Western Blot. For reducing conditions, add 1mM β-mercaptoethanol or DTT (fresh) and boil for 5 minutes prior to loading on gel.

Literature

References:

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