SUMO2 Conjugation Kit

Catalog Number K-715

This kit contains reagents sufficient for 10 x 20 µL reactions.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION PAGE

MANUFACTURED BY:

Boston Biochem, Inc.
840 Memorial Drive
Cambridge, MA 02139, USA
TEL: (617) 576-2210 FAX: (617) 492-3565
E-MAIL: techsupport@bostonbiochem.com

DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@bio-techne.com

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@bio-techne.com

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

The ubiquitin-like SUMO2 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). This isoform is known to form poly-SUMO chains on substrates via isopeptide bond formation between the C-terminal glycine of one SUMO2 and the lysine-ε-amino group of K11 of the preceding SUMO2. The heterodimeric SAE1/SAE2 complex (38 and 70 kDa respectively) uses ATP to adenylate the C-terminal glycine residue of a SUMO protein; in a second step a thioester bond is formed between SUMO and a cysteine residue within the SAE2 subunit. Following this activation, SUMO is transferred to Cys93 of Ubc9. Ubc9 (also known as UBE2I) is a member of the E2 family and is specific for the conjugation of SUMO to a variety of target proteins. This E2 is unusual in that it sometimes interacts directly with protein substrates that are SUMOylated, and can play a role in substrate recognition. SUMOylated substrates are often 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.

TECHNICAL HINTS

- The included protocol is provided as a general guideline for a 20 µL volume reaction. The recommended substrate volume of 5 µL may be altered to accommodate experimental design, and the total reaction size may be scaled up or down depending on individual requirements. Individual results may vary with different conditions and substrates, it is up to the end-user to optimize the reaction. Available controls for SUMOylation reactions include a biotinylated negative control peptide (SP-305), a positive control peptide (SP-300) or E2-25K protein (E2-603). See data below. The kit protocol suggests that reactions be terminated with SDS-PAGE Sample Buffer; therefore, proteins are denatured and typically not suitable for further enzymatic manipulation. If SUMOylated samples are to be used in downstream applications the reactions may be terminated by adding EDTA and DTT (10-20 mM final) if compatible with experimental design.
- When complex samples (such as lysates or partially purified proteins from eukaryotic cells) are used as a source of substrate, the user may want to consider adding SUMO-Vinyl Sulfone (UL-769) to reactions to counteract the presence of Sentrin proteases that may be present. It such instances the suggested starting concentration of SENP inhibitor is 1-5 µM final, and the volume of water in the reactions should be adjusted to compensate for the added inhibitors.
- Further information is available via techsupport@bostonbiochem.com

MATERIALS PROVIDED & STORAGE CONDITIONS

Kit contains reagents sufficient for 10 x 20 µL reactions.

COMPONENT	VOLUME	STORAGE OF COMPONENTS
10X SUMO E1 enzyme	20 µL	Store at -80 °C.* Avoid multiple freeze-thaw cycles.
10X SUMO2	20 µL	
10X Ubc9 (UBE2I)	20 µL	
10X Reaction Buffer	20 µL	
10X Mg^{2+}-ATP Solution	20 µL	

*Provided this is within the expiration date of the kit.

OTHER MATERIALS REQUIRED

- **Waterbath or heating block:** 37 °C
- **Reaction tubes:** 0.5 mL or 1.5 mL polypropylene, microcentrifuge compatible
- **dH_2O:** Sterile
- **Sentrin-specific Protease (SENP) Inhibitor**
- **EDTA:** 0.5 M solution; optional
- **Dithiothreitol (DTT):** 1M in dH_2O (Pierce #20290) or similar; optional
- **SDS-PAGE Sample Buffer:** optional

ASSAY PROTOCOL

Reagent Preparation

1. Quickly thaw all protein reagents and buffers by gently and continuously swirling tubes in a lukewarm water bath (≤ 30°C). Alternatively, tubes may be thawed with a rapid back-and-forth rolling motion between palms of hands. Do not heat tubes for an extended period of time. Do not vortex or shake vigorously.

2. When completely thawed, **gently** tap tubes to make sure components are well mixed and then briefly spin in a microcentrifuge (5 seconds) to collect contents in bottom of tubes.

3. Immediately ice components. Entire process from steps 1-2 should be accomplished in ≤ 5 minutes.

4. It is **strongly recommended** that each reagent be divided into smaller aliquots to minimize the number of freeze-thaw cycles of kit components. Avoid multiple freeze-thaw cycles to maximize kit performance. Rapidly re-freeze any aliquoted materials in dry ice bath.

Reaction Assembly

1. Prepare reactions on ice in 0.5 or 1.5 mL polypropylene tubes using the following volumes and order of addition:
   a. 5 µL dH_2O
   b. 2 µL 10X Reaction Buffer. Mix gently following addition.
   c. 5 µL Substrate protein at 10-40 µM stock (provided by user, suggested final concentration of 2.5-10 µM substrate)
   d. 2 µL 10X SUMO E1 enzyme
   e. 2 µL 10X Ubc9/UBE2I (E2) enzyme
   f. 2 µL 10X SUMO2
2. Any pre-reaction incubations may be done at this point (*e.g.* treatment of complex lysates with SENP inhibitors). Addition of Mg^{2+}-ATP in the next step will start the reaction.
3. Add 2 µL of 10X Mg^{2+}-ATP solution. Mix by gently pipetting up and down 2-3 times. For negative control reactions, omit ATP addition and replace with 2 µL dH_2O.
4. Spin tubes to collect contents and place reactions in 37 °C water bath.
5. After 30-60 minutes, terminate reactions with addition of 5 µL 5X Loading Buffer (SDS-PAGE sample buffer) and 1 µL 1M DTT. (An initial time course is recommended to determine the optimal incubation time for efficient substrate conjugation.)
6. Heat reactions to 90 °C for 5 minutes.

SAMPLE DATA

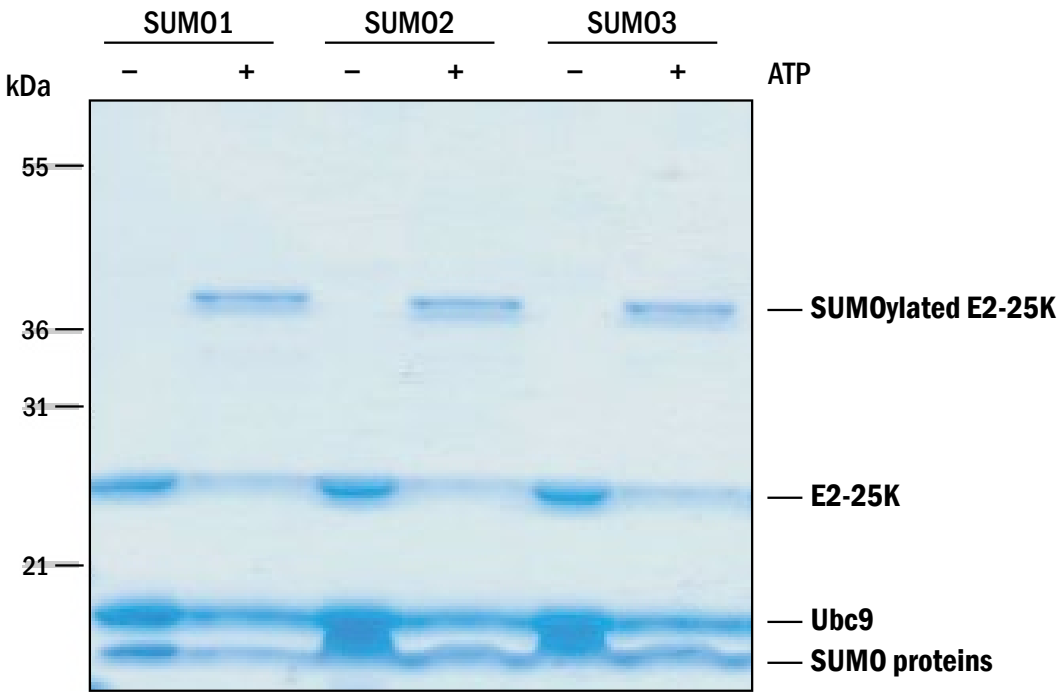

Figure 1: Modification of E2-25K (E2-603) by SUMO1, SUMO2, and SUMO3 proteins. 20 µL reactions were assembled as described in the protocol using 5 µM E2-25K as a substrate. Reactions were incubated at 37 °C for 60 minutes then terminated with SDS-PAGE Sample Buffer + 10 mM DTT. Proteins were separated by reducing 4-20% SDS-PAGE and visualized with Coomassie Blue stain.

For research use only. Not for use in diagnostic procedures.

SAMPLE DATA *CONTINUED*

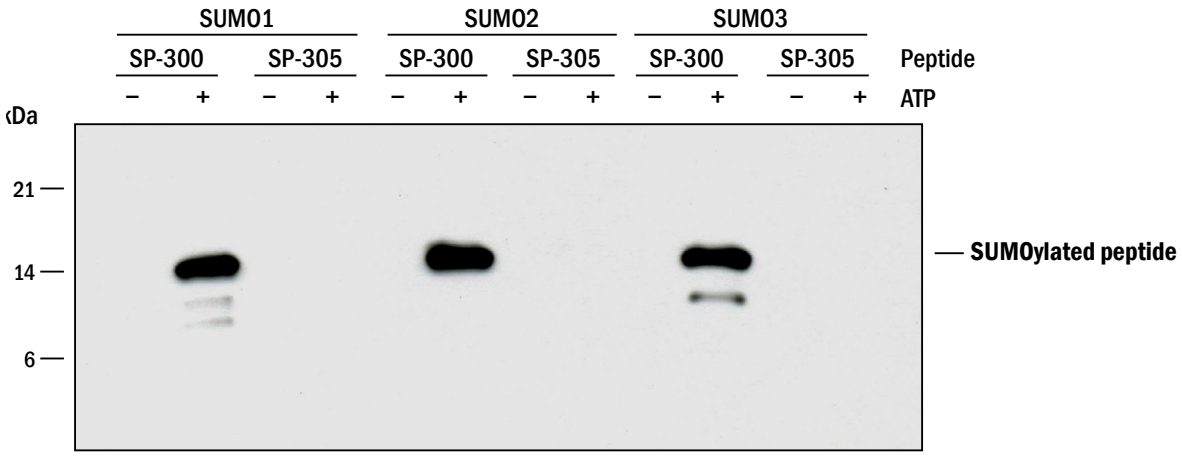

Figure 2: Western blot detection of consensus peptide (SP-300) modification by SUMO proteins. 20 µL reactions were assembled as described in the protocol using 50 µM peptides as substrates. Reactions were incubated at 37 °C for 60 minutes then terminated with SDS-PAGE Sample Buffer + 10 mM DTT. Peptide SP-300 contains a SUMO consensus motif and SP-305 is a scrambled version (negative control). SUMOylated peptides were detected with avidin-HRP and ECL substrate, while unmodified peptides are not visible because they migrate off the gel.

REFERENCES

Lapenta V., *et al.* (1997) Genomics **40**:362-366.

Gill G. (2004) Genes.Dev. **18**:2046-2059.

Meluh P.B. and Koshland D. (1995) Mol. Biol. Cell **6**: 793-807.

Pichler A. *et al.* (2005) Nat. Struct. Mol. Biol. **12**:264-269.

Saitoh H. and Hinchey J. (2000) J.Biol. Chem. **275**:6252-6258.

Su H.L. and Li S. S.-L. (2002) Gene **296**: 65-73.

Subramanian L., *et al.* (2003) J.Biol. Chem. **278**:9134-9141.

Yeh E.T.H., *et al.* (2000) Gene **248**:1-14.