
In vitro Sumoylation Assay Kit (Cat No. SA-001/SB-001)

I. Reagents*

- A. 10x Reaction Buffer (200 μ l): 0.2 M Hepes-NaOH, pH 7.4, 50 mM MgCl₂, 20 mM ATP, 0.5 mM PMSF, and 1 mg/ml BSA
- B. SUMO-1 (50 μ l, 1 mg/ml)
- C. GST-Ubc9 (50 μ l, 0.4 mg/ml)
- D. SAE1/SAE2 (50 μ l, 1 unit/ μ l)
- E. Control peptide (50 μ l, 0.5 mg/ml): GST-IkB α ^a (SA-001) or flag-tagged GST-RHA₁₃₇^b (SB-001)
- F. 5x SDS sample buffer (250 μ l)

**All reagents should be completely thawed, mixed by vortex, and briefly centrifuged. They should be always kept on ice during the preparation of reaction mixture described below and returned to below -70°C immediately after use.*

II. *In vitro* Sumoylation Assay

Step 1. Prepare reaction mix (20 μ l/reaction)^c in the following order and ratio.

Reagent A	2 μ l
Reagent B	1 μ l
Reagent C	1 μ l
Reagent D	1 μ l
Reagent E	1 μ l

(or peptide of your interest)
Deionized H₂O^d to a final volume of 20 μ l.

Step 2. Mix well by vortex and centrifuge for 10 sec.

Step 3. Incubate at 37°C for 120 min.

Step 4. Add 5 μ l of **Reagent F** to each reaction.

Step 5. Incubate for 3 min at 95°C.

Step 6. Resolve the reaction mixture by SDS-PAGE (7.5-10%).

Step 7. Following gel electrophoresis, transfer proteins from the gel to nitrocellulose^d or PVDF^d membrane.

Step 8. Visualize the peptide and its sumoylated form by the standard ECL-Western blot analysis using antibodies^e that recognize the target peptide.

a. *MCB 2, 233-239 (1998).*

b. *JMB 341, 15-25 (2004).*

c. *For reaction in smaller volume, proportionally decrease the amount of reagents described in this manual.*

d. *Not provided.*

e. *Necessary immunological reagents are not included. For the detection of GST-IkB α and GST-RHA137, IkB α -specific antibodies and M2 (sigma) antibodies, respectively, should be used. Do not use antibodies recognizing GST.*