SUMOylation kit

Market leading kit for generation of SUMOylated proteins *in vitro*.

This kit provides a means of generating SUMOylated proteins *in vitro*, by covalent linkage of the carboxy-terminal of SUMO-1, -2 or -3 to specific lysine residues on the target protein *via* isopeptide bonds, using the SUMOylation enzyme cascade. A short sequence containing the consensus ?-K-X-D/E (where lysine is the amino acid modified, ? is a large hydrophobic residue and X is any amino acid residue) is thought to be necessary for this *in vitro* protein SUMOylation to occur, however SUMOylation has also been observed in cases where the consensus site is not conserved. A control target protein is provided together with all other necessary components. SUMO specific antibodies are provided for detection of SUMOylated proteins *via* SDS-PAGE and Western blotting. Provides sufficient material for 20 x 20µL reactions. Suggested uses: For SUMO-modification of specific proteins *in vitro*, To demonstrate that novel proteins are potential targets for SUMOylation under *in vitro* conditions, To generate substrates for deSUMOylating enzymes, such as SENP1 and SENP2, To test proteins for SUMO E3 ligase activity.

Citations: 59 View Online »

Ordering Information

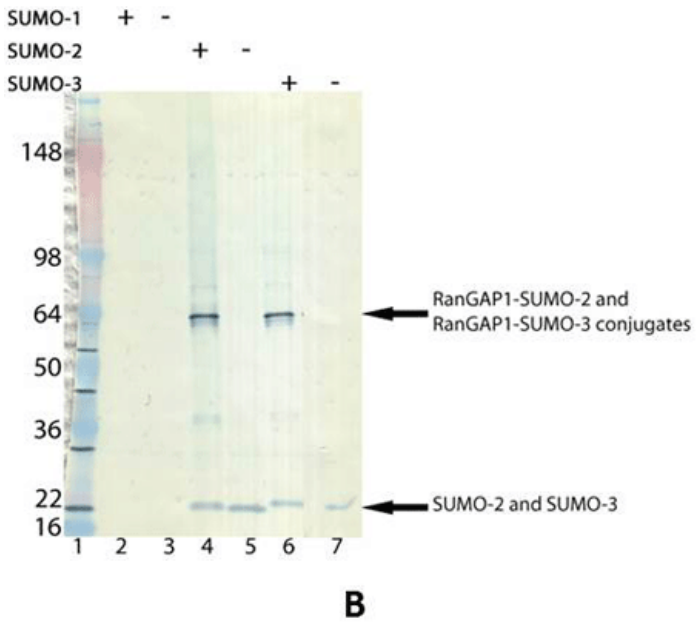
Order Online »

BML-UW8955-0001	20 tests

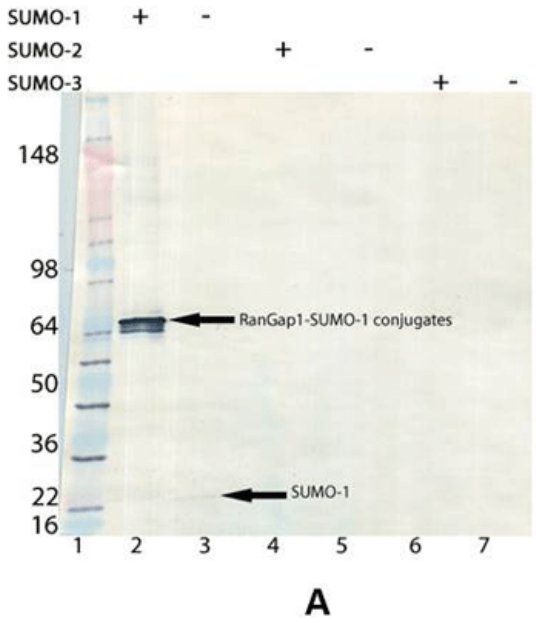
Manuals, SDS & CofA

View Online »

- Fastest assay time on the market, just under 1.5 hours
- Simple 4 step assay: Mix, Incubate, Quench and Assay
- High specificity, high throughput capacity
- Amenable to analysis via Western blotting or proteomic methods



Western Blot of SUMOylation Assays for control RanGAP1 target protein. Assays set-up and run as described in “Assay Protocol”. SUMOylated proteins were detected by Western Blotting as described in “Analysis by Western Blotting”. B: detected by SUMO-2/3, pAb (BML-PW9465).



Western Blot of SUMOylation Assays for control RanGAP1 target protein. Assays set-up and run as described in “Assay Protocol”. SUMOylated proteins were detected by Western Blotting as described in “Analysis by Western Blotting”. A: detected by SUMO-1, pAb (BML-PW8330)

Handling & Storage

Use/Stability	Unopened kit should be stored at -80°C to ensure stability and activity. After opening, The SUMO antibody solutions (BML-PW8330 and BML-PW9465) can be stored at -20°C or -80°C. Other components should be stored at -80°C.
Handling	Avoid freeze/thaw cycles.
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application Notes

Uses:

1. SUMO-modification of specific proteins *in vitro*. Allow investigation of the effect SUMOylation has on enzyme function, stabilisation, protein:protein interactions and, hence, it's role in regulation of cellular processes, such as the p53 tumour repressor and NF-κB pathways.
2. Demonstrate novel proteins are potential targets for SUMOylation under *in vitro* conditions. Starting point for examining the role SUMOylation of a protein might play *in vivo*.
3. Generate substrates for deSUMOylating enzymes, such as SENP1 (Prod. No. BML-UW9760) and SENP2 (Prod. No. BML-UW9765).
4. Test proteins for SUMO E3 ligase activity: does it facilitate or enhance SUMOylation of specific target proteins, particularly under conditions/enzyme concentrations that more closely represent those *in vivo.*
5. Addition of known SUMO E3 ligase to facilitate/enhance target protein SUMOylation, particularly under conditions/enzyme concentrations that more closely represent those *in vivo* (e.g. RANBP2 [Prod. No. BML-UW9455], shown to be a ligase for SP100 SUMOylation).
6. SUMOylation of proteins in cell lysates or crude fractions/preparations to facilitate investigation of their role/function in complex solutions.
7. Demonstrate SUMOylation of known proteins in specific lysates (confirm with target protein specific antibodies).
8. Use of cell lysate or crude fractions/preparations as source of SUMO E3 ligases to facilitate SUMOylation of purified target proteins in the presence of SUMOylation kit components.

Note: Protocol provided for application 1. Assay set-up can be readily modified for alternative applications by inclusion, omission or substitution of specific components.

Contents	**20x SUMO Activating Enzyme Solution (SUMO E1):** SUMO activating enzyme E1 (human), (recombinant), (Prod. No. BML-UW9330), 20 µl. **20x SUMO Conjugating Enzyme Solution (SUMO E2):** Ubc9 (human), (recombinant) (untagged), (Prod. No. BML-UW9320), 20 µl. **20x SUMO Enzyme Solutions (SUMO-1, SUMO-2, SUMO-3):** SUMO-1 (human), (reccombinant) (His-tag), (Prod. No. BML-UW9195), 20 µl; SUMO-2 (human), (reccombinant) (His-tag), (Prod. No. BML-UW9205), 20 µl; SUMO-3 (human), (reccombinant) (His-tag), (Prod. No. BML-UW9215), 20 µl. 10x SUMOylation Buffer (Prod. No. BML-KW9890), 40 µl **20x Control RanGAP1 SUMOylation Target Protein Solution:** RanGAP1 fragment (human), (recombinant) (GST-tag), (Prod. No. BML-UW9755), 20 µl. **20x Mg-ATP Solution** (Prod. No. BML-EW9805), 25 µl **SUMO Antibody Solutions:** SUMO-1 (human) (NT), polyclonal antibody, (Prod. No. BML-PW8330), 25 µl; SUMO-2/3 (human) (NT), polyclonal antibody, (Prod. No. BML-PW9465), 25 µl.
Quantity	Sufficient for 20 assays.

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