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: 58

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Ordering Information

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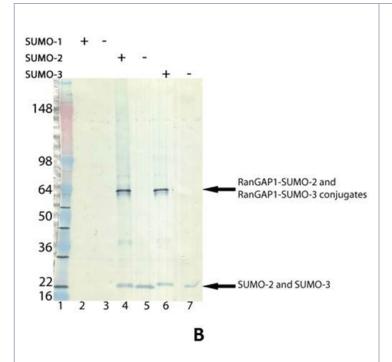
BML-UW8955-0001

20 tests

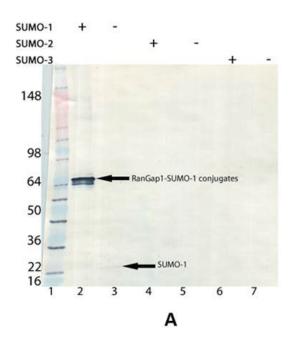
Manuals, SDS & CofA

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- 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-kB 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 μ l.

20x SUMO Conjugating Enzyme Solution (SUMO E2):

Ubc9 (human), (recombinant) (untagged), (Prod. No. BML-UW9320), 20 μ l.

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 μ l.

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 μ l.

Sufficient for 20 assays.

Quantity

Last modified: September 3, 2025



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