

## Product Information

## Anti-SUMO 2/3 Magnetic Beads

Magnetic agarose, suspension

**SAE0221**

Storage Temperature: 2-8 °C

Synonyms: Anti-HSMT3, Anti-MGC117191, Anti-SMT3 homolog 2, Anti-SMT3 suppressor of mif two 3 homolog 2, Anti-SMT3B, Anti-SMT3H2, Anti-sentrin 2, Anti-small ubiquitin-like modifier 2, Anti-small ubiquitin-related modifier 2, Anti-ubiquitin-like protein SMT3B, Monoclonal Anti-Sumo-2/3, SUMO 2/3

### Product Description

In situations where protein-specific antibodies are unavailable, epitope tags enable researchers to study protein topology, characterize and identify new proteins, protein complexes, and associated proteins, even if the proteins of interest are in low abundance or are poorly immunogenic.<sup>1-4</sup>

Monoclonal anti-SUMO2/3 antibody, derived from the hybridoma 3H12 produced by the fusion of mouse myeloma cells and splenocytes from rat immunized with a human SUMO. The isotype is determined using a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents. Monoclonal Anti-SUMO2/3 recognizes specifically an epitope located on SUMO tagged fusion proteins expressed in transfected mammalian cells or produced by *in vitro* translation.<sup>5</sup> The antibody reacts specifically with SUMO2/3-tagged fusion proteins and may be used for the immunoprecipitation or immunoaffinity purification.

Small ubiquitin-like modifier (SUMO) was identified as a post-translational protein modifier. It functions in a manner similar to ubiquitin, bounding to target proteins as part of a post-translational modification system. However, unlike ubiquitin, which targets proteins for degradation, SUMOylation is involved in a variety of cellular processes, such as nuclear transport, transcriptional regulation, apoptosis, and protein stability.

There are four members in the SUMO family, of which SUMO 1–SUMO 3 are ubiquitously expressed, whereas SUMO 4 is mainly expressed in the lymph system. SUMO 2 and SUMO 3 are 97% identical (thus referred to as SUMO 2/3) but share only 50% sequence identity with SUMO 1.<sup>6</sup> SUMO 2/3 localize to centromeres and condensed chromosomes, together with this, it was found to have protein targets, signaling properties and functions that are unique from those of SUMO-1.<sup>7-8</sup> Nevertheless, *in vivo*, SUMO 2/3 were able to compensate for the lack of SUMO 1, suggesting redundancy is an important cellular strategy for maintaining SUMOylation levels above critical threshold.<sup>9</sup>

SUMO-tag provide a method to localize gene products in a variety of cell types, study the topology of proteins and protein complexes, identify associated proteins, and characterize newly identified, low abundance or poorly immunogenic proteins when protein specific antibodies are not available.

Anti-SUMO 2/3 Magnetic beads are prepared with an affinity purified anti-SUMO 2/3 antibody, produced in mouse (SAB4200190). The antibody is conjugated to activate Magnetic beads at a 4:1 protein to beads ratio.

Anti-SUMO 2/3 magnetic beads are useful in the purification of a SUMO 2/3 fusion protein in bacteria or in transfected mammalian cells with a SUMO2/3 fusion protein expressing vectors.

## Reagent

The Anti SUMO 2/3 Magnetic beads product is provided as suspension containing 50% Beads, in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the safety data sheet for information regarding hazards and safe handling practices.

## Product Profile

- Binding capacity: 600-7000  $\mu\text{g}$  of SUMO2/3-tagged fusion protein per 1 mL of settled resin
- Elution capacity: 250-5000  $\mu\text{g}/\text{mL}$  settled resin

## Storage/Stability

For continuous use and extended storage, store at 2-8 °C. Do not freeze.

## Notes

- To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing, or using a rotating platform.
- The following General Procedure is written for use of 20  $\mu\text{L}$  of the anti-SUMO 2/3 magnetic agarose beads, and an estimated bead capacity of 60  $\mu\text{g}$  of recombinant SUMO 2/3 protein.
- Our suggestion is to add enough cell lysate where 60  $\mu\text{g}$  of the SUMO 2/3-tagged protein is expected to be present.
- Each sample will differ, depending on the individual researcher's system, and determination or estimation of the degree of SUMO 2/3 tagged protein in the lysate sample.
- A method like SDS-PAGE analysis can be performed on an aliquot of the lysate, with estimated quantitation of lysate protein bands against known amounts of defined protein standards in a separate gel lane.

## Procedures

To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.

1. Prewashing: Add 20  $\mu\text{L}$  of Anti SUMO 2/3 Magnetic beads into a 1.5 mL microcentrifuge tube.
2. Place the tube into a magnetic stand to collect the beads against the side of the tube.
3. Remove and discard the supernatant. Wash with 200 mL 0.01 M PBS, repeat washing 3-5 times.
4. Apply the sample which contains the estimated amount of 60  $\mu\text{g}$  SUMO 2/3 tagged protein. Gently pipette mix. Incubate for 60 minutes at 37 °C using a rotating platform.
5. Save the flowthrough for calculating the binding capacity.
6. Wash with 0.01 M PBS until the absorbance at 280 nm is minimal.
7. Elution of SUMO 2/3 tagged proteins may be done by incubation with 80 mL sample buffer X1 for 5 min at 95 °C.

## Notes

- To obtain the best results in different techniques and preparations, we recommend on determining optimal working concentration by titration test.
- Binding capacity and elution capacity may vary, depending on the characteristics of the SUMO 2/3-tagged fusion proteins. For optimal results, it is recommended to try different elution buffers.

## References

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