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This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

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Product no AS10 847

Anti-Sml1 | Suppressor of Mec1 lethality

Product information

Immunogen	KLH-conjugated synthetic peptide derived from known *S.cerevisie* Sml1 sequence. Gene ID: 854945
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl of sterile water
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	11.83 | 11-12 kDa
Confirmed reactivity	*Saccharomyces cerevisiae*
Predicted reactivity	*Saccharomyces cerevisiae*
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	Cell preparation for western blot: cells were harvested by centrifugation (4000 x g , 6 min, 4°C). Supernatant was discarded and cells were resuspended in 500 µl cold TCA buffer (20 mM Tris, pH 8, 50 mM ammonium acetate, 2 mM EDTA, 1 tablet/10 ml of Complete Mini Protease inhibitor cocktail with EDTA (Roche Diagnostics GmbH)). 500 µl 0.5 mm Zirconia/Silica Beads (BioSpec Products, Inc, 11079105z) and 500 µl cold 20% TCA was added. Samples were vigorously vortexed twice for 30 s (kept on ice in between), 750 µl from the liquid phase was transferred into a fresh Eppendorf tube. Samples were centrifuged for 10 min (20000 x g, 4°C). The pellet was resuspended in 300 µl TCA-Laemmli buffer and boiled for 10 min at 100°C.
Selected references	Cerritelli et al. (2020). High density of unrepaired genomic ribonucleotides leads to Topoisomerase 1-mediated severe growth defects in absence of ribonucleotide reductase. Nucleic Acids Res Corcoles-Saez et al. (2019). Essential Function of Mec1, the Budding Yeast ATM/ATR Checkpoint-Response Kinase, in Protein Homeostasis. Dev Cell. 2018 Aug 20;46(4):495-503.e2. doi: 10.1016/j.devcel.2018.07.011. Garbacz et al. (2019). The absence of the catalytic domains of Saccharomyces cerevisiae DNA polymerase strongly reduces DNA replication fidelity. Nucleic Acids Res. 2019 Jan 30. doi: 10.1093/nar/gkz048. Golla et al. (2017). A systematic assessment of chemical, genetic, and epigenetic factors influencing the activity of anticancer drug KP1019 (FFC14A). Oncotarget. 2017 Sep 30;8(58):98426-98454. doi: 10.18632/oncotarget.21416. Dmowski et al. (2017). Mutations in the Non-Catalytic Subunit Dpb2 of DNA Polymerase Epsilon Affect the Nrm1 Branch of the DNA Replication Checkpoint. PLoS Genet. 2017 Jan 20;13(1):e1006572. doi: 10.1371/journal.pgen.1006572. Mertz et al. (2015). Colon cancer-associated mutator DNA polymerase variant causes expansion of dNTP pools increasing its own infidelity. Proc Natl Acad Sci U S A. 2015 May 12;112(19):E2467-76. doi: 10.1073/pnas.1422934112. Epub 2015 Mar 31. Singh et al. (2014). Anti-cancer drug KP1019 modulates epigenetics and induces DNA damage response in Saccharomyces cerevisiae. FEBS Lett. 2014 Feb 20. pii: S0014-5793(14)00137-9. doi: 10.1016/j.febslet.2014.02.017. Azad et al. (2013). Depletion of Cellular Iron by Curcumin Leads to Alteration in Histone Acetylation and Degradation of Sml1p in Saccharomyces cerevisiae. PLoS One, March 8. Poli et al. (2012).dNTP pools determine fork progression and origin usage under replication stress. The EMBO J. January 2012, 1-12.

Application example

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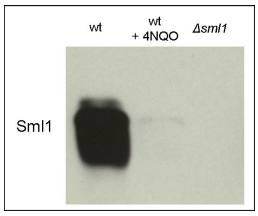

10 µl total protein from 9.25 x 107 cells of *Saccharomyces cerevisiae* extracted with 20% TCA as described below were separated on 20% SDS-PAGE and blotted 1.5h (0.5 A) to a nitrocellulose membrane (Whatman PROTRAN BA 85, 0.45 µm). Blots were blocked with 5% non-fat dry milk in TBST for 1.5h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 5 000 overnight at 4C° with agitation. The antibody solution was decanted and the blot was washed 3 times for 10 min in TBST at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated from Agrisera, (AS09 602) diluted to 1:50 000 for 1h at RT with agitation. The blot was washed as above and developed for 3 min with chemiluminescent detection reagent. Exposure time was 30 seconds.

Courtesy Dr. Andrei Chabes**,** Umeå University, Sweden