abcam

Product datasheet

Recombinant Human UBE2I / UBC9 protein ab3803

画像数 3

製品の概要

製品名	Recombinant Human UBE2I / UBC9 protein
タンパク質長	Full length protein

製品の詳細

由来	Recombinant
由来	Escherichia coli
アミノ酸配列	
生物種	Human
分子量	18 kDa

特性

Our Abpromise guarantee covers the use of **ab3803** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

生理活性	The final fraction of enzyme contains a single polypeptide band of 18 kDa.
アプリケーション	Western blot Functional Studies
製品の状態	Liquid
備考	This product can be used as part of an assay for sumoylation activity. Human Aos 1 + Uba 2 (ab3804), Ubc 9 (ab3803) and Sumo 1 (ab3801) can be used to promote in vitro sumoylation of a sumoylation marker (human Topoisomerase I protein fragment) (ab3828). The reaction products can be detected using our Sumo 1 (ab3819 and ab3824) and Topoisomerase I (ab3825) antibodies. Sumoylation assays are carried out in a final volume of 20µl in reaction conditions (20 mM Hepes pH 7.5, 5mM MgCl2, 2mM ATP). Sumoylation Protocol: 1. Prepare a suitable purified substrate protein. (For the control, use 2µl Topoisomerase I marker for each reaction.) 2. In each reaction, add 4µl E2 to substrate first, then 2µl Sumo 1, 2µl 10x reaction buffer, 2µl E1. Finally, add H2O to bring up to 20µl. We would recommend adding fresh 2mM ATP to be sure that sufficient energy is supplied. 3. The best reaction concentration of proteins is as following: Aos 1 + Uba 2: 7.5µg/ml. Ubc 9: 50µg/ml. SUMO 1: 50µg/ml. For the control assay we recommend running the assay at 37ºC for 30-60 minutes. 4. Detect the reaction

products by Western blot using a suitable antibody. For the control reaction use 1/1000 dilution of the supplied Topoisomerase I antibody. Four sumoylated bands should be seen on the gel for the control reaction. This assay has been shown to work with crude extracts. Be aware that Uba 2 contains his-rich regions which might cross-react with antibodies against the 6x-His epitope tag. During western analysis with anti-6x-His antibodies, Uba 2 at 80 kDa might be shown.

The final fraction of enzyme contains a single polypeptide band of 18 kDa.

前処理および保存

保存方法および安定性

Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

10 mM Tris-Cl, pH 7.5, 100 mM NaCl, 100 mM imidazole, 0.5 mM PMSF, 1 mM DTT, and 10 % glycerol

関連情報

機能

Accepts the ubiquitin-like proteins SUMO1, SUMO2, SUMO3 and SUMO4 from the UBLE1A-UBLE1B E1 complex and catalyzes their covalent attachment to other proteins with the help of an E3 ligase such as RANBP2 or CBX4. Necessary for sumoylation of FOXL2 and KAT5. Essential for nuclear architecture and chromosome segregation.

組織特異性

Expressed in heart, skeletal muscle, pancreas, kidney, liver, lung, placenta and brain. Also expressed in testis and thymus.

パスウェイ

Protein modification; protein sumoylation.

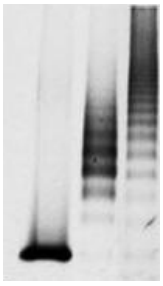
配列類似性

Belongs to the ubiquitin-conjugating enzyme family.

細胞内局在

Nucleus. Cytoplasm. Mainly nuclear. In spermatocytes, localizes in synaptonemal complexes. Recruited by BCL11A into the nuclear body.

画像



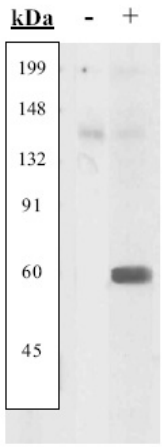
Sumoylation assay

Topo I sumoylation

Left: Human topo I (S^{35}Met-labeled) control.

Right: Sumoylated human topo I (S^{35}Met-labeled)

(7.5 µg/ml E1, 50 µg/ml E2, 50 µg/ml sumo1, 37ºC 30min).



Western blot - UBE2I / UBC9 full length protein (ab3803)

Extracts prepared from CEF cells not transfected (-) or transfected (+) with wild-type Src were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to nitrocellulose. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4°C, then were incubated with 1.0 µg/mL anti-Src pan antibody. After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase and the signal was detected using the Tropix WesternStar method.

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