abcam

Product datasheet

SUMOylation Assay Kit ab139470

14 References 2 图像

概述

产品名称	SUMOylation Assay试剂盒
样品类型	Purified protein
检测类型	Enzyme activity
产品概述	SUMOylation Assay Kit (ab139470) 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 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. This kit provides sufficient material for 20 x 20µL reactions. Suggested uses for the SUMOylation assay kit include: 1) SUMO-modification of specific proteins in vitro. Allow investigation of the effect SUMOylation has on enzyme function, stabilization, protein:protein interactions and, hence, it's role in regulation of cellular processes, such as the p53 tumor repressor and NF-$_{K}$B 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 and SENP2. 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, 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) Assay 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.

说明

The mechanism for SUMO conjugation is analogous to that of the ubiquitin system, relying upon utilization of E1, E2 and E3 cascade enzymes. SUMO modification of target proteins is involved in nuclear protein targeting, formation of sub-nuclear complexes, regulation of transcriptional activities and control of protein stability.

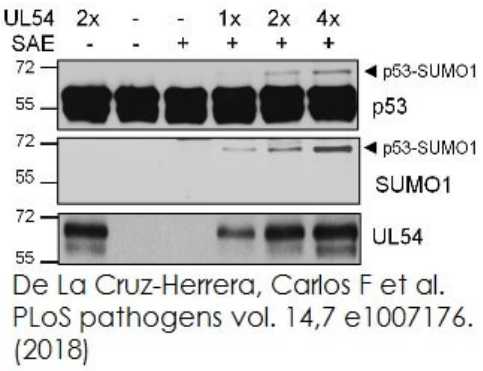
A short sequence containing the consensus Ψ-K-X-D/E (where Lysine is the modified amino acid, Ψ is a large hydrophobic residue and X is any amino acid residue) is thought to be necessary for the protein SUMOylation to occur.

性能

存放说明 Please refer to protocols.

组件	20 tests
10x SUMOylation Buffer	1 x 40µl
20X Mg-ATP Solution	1 x 25µl
20x RanGAP1 Fragment (Human, Recombinant)	1 x 20µl
20X SUMO Activating Enzyme E1	1 x 20µl
20X SUMO1 (Human, Recombinant)	1 x 20µl
20X SUMO2 (Human, Recombinant)	1 x 20µl
20X SUMO3 (Human, Recombinant)	1 x 20µl
20x Ubc9 (SUMO E2) (Human, Recombinant)	1 x 20µl
SUMO1 Rabbit Polyclonal Antibody	1 x 25µl
SUMO2/3 Rabbit Polyclonal Antibody	1 x 25µl

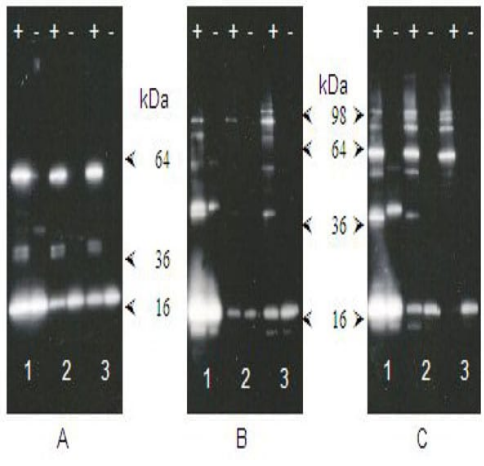
图片



Functional Studies - SUMOylation Assay Kit (ab139470)

De La Cruz-Herrera, Carlos F et al., PLoS pathogens? vol. 14,7 e1007176., Fig 8, doi:10.1371/journal.ppat.1007176

In vitro SUMOylation assays were performed using Abcam SUMOylation Assay Kit with SUMO1 (left panels) and E.coli purified full length p53 as a substrate.



Western Blot of SUMOylation Assays for RANGAP1 control target and SP100/p53 target proteins.

Assays set-up and run as described in “Assay Protocol”. SUMOylated proteins were detected by Western Blotting on SUMOylation assays containing A: RANGAP1, B: p53 and C: SP100 target proteins with 1: SUMO1, 2: SUMO2 and 3: SUMO3 substrates using the appropriate SUMO antibody assays. SUMOylation assays set-up and run as described in “Assay protocol”.

Results demonstrate the formation of SUMOylated target proteins of the expected size in all ATP containing reactions. The absence of such conjugates in –ve control reactions demonstrates that their formation is ATP dependent (required for E1 activation) and hence derived from the SUMO cascade.

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