V. 1.2

SOS1 exchange domain (564-1049) protein
(Human recombinant)
Cat. # CS-SOS1-A
Lot: 014 **Amount 1 x 100 µg**
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material
The human SOS1 exchange domain (564-1049) protein (SOS1-ExD) has been produced in a bacterial expression system. The recombinant protein contains a 6 histidine tag (His-tag) at its amino terminus. The approximate molecular weight of the SOS1-ExD protein is 61 kDa. The protein is supplied as a lyophilized white powder.

Storage and Reconstitution
The protein should be reconstituted to 50 µM (3.03 mg/ml) by the addition of 33 µl of distilled water. The protein will be in the following buffer; 17 mM Tris pH 7.5, 17 mM NaCl, 0.2 mM $MgCl_2$, 2.3% sucrose, 0.3% dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution should be aliquoted into "experiment sized" aliquots and snap frozen in liquid nitrogen. The protein can be stored at -70°C for 6 months. Avoid repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C for 1 year when stored desiccated (<10% humidity).

Purity
Protein purity is determined by scanning densitometry of Coomassie blue stained protein on a 4-20% polyacrylamide gradient gel. The SOS1-ExD protein was determined to be >90% pure. (see Figure 1).

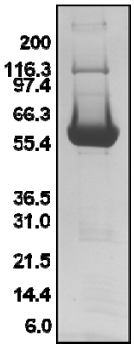

Figure 1. His-hSOS1 (564-1049) Protein Purity Determination. A 20 µg sample of recombinant SOS1-ExD protein (molecular weight approx. 61 kD) was separated by electrophoresis in a 4-20% SDS-PAGE system. The protein was stained with coomassie blue. Protein quantification was determined using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay
The biological activity of SOS1-ExD can be determined from its ability to catalyze nucleotide exchange on Ras isoforms (N,H, K) using the nucleotide exchange assay with Bodipy-GDP and excess GDP or GTP. Rac1 protein is pre-loaded with Bodipy-FL-GDP by adding excess EDTA e.g. 0.7 mmol EDTA per mmol Mg2+ ions present in the reaction. This sub-stock solution is then used in a dissociation assay format which indicates competition for exchange site with unlabeled nucleotide. The reaction is monitored by fluorescence measurement at 485nm Ex / 535nm Em. Stringent quality control ensures that the exchange rate of Bodipy-GTP or mant-GTP is enhanced at least five fold in the presence of 0.8 µM SOS1-ExD.

Reagents, Materials and Equipment
1. SOS1 ExD protein (Cat. # CS-SOS1)
2. Ras protein (Cat. # RS01 (H-Ras), RS02 (N-Ras), RS03 (K-Ras), RS04 (K-Ras-G12V), or RS05 (R-Ras).
3. Exchange buffer 2 (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM DTT, 2 mM EDTA, 100 µg/ml BSA, and 0.75 µM Bodipy-GDP or Mant-GDP), note - make fresh.
4. 50 mM MgCl2 in 20 mM Tris-HCl pH 7.5, 50 mM NaCl.
5. 5 mM GTP in 20 mM Tris-HCl pH 7.5, 50 mM NaCl..
6. 96-well plate fluorescence spectrophotometer.
7. Fluorescence half area low-binding black 96-well plate (Corning Cat. # 3686).

Method: over page.

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Method

1. Place SOS1 ExD vial on ice and dilute to 0.30 µg/µl (8 µM) with ice cold Exchange Buffer.

2. Place Ras vial on ice and dilute to 1.25 µg/µl (50 µM) with ice cold Exchange Buffer..

3. Add the following components together in a fresh 15 ml Falcon tube and mix well by pipetting or gentle vortex:

Component	per well
Exchange Buffer	75 µl
50 µM Ras	5 µl
8 µM SOS1	10 µl

Note: For a total mixture volume, multiply the volume of reagents per well by the number of wells in the experiment, plus add 20% volume for pipetting losses.

5. Incubate for 20 min at room temperature (RT).

6. Lock in the nucleotide by adding 10 µl (per well) of 50 mM $MgCl_2$.

7. Set up the fluorimeter with Excitation wavelength at 485 nm +/-20 nm and emission wavelength at 535 nm +/- 20 nm at RT.

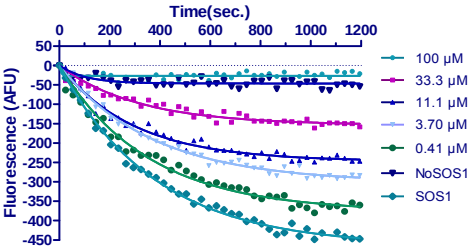
8. Aliquot the pre-loaded mixture to the assigned wells and place the plate in the fluorimeter.

9. After 5 cycles (150 seconds), place the program on Hold or Pause, and remove the plate.

10. Pipette 10 µl of a) 5 mM GTP solution, b) a small compound, c) a test protein, d) 4 mM EDTA (+ve exchange control) or e) Dilution Buffer (negative control) in respective wells and immediately pipette up and down twice and resume reading for 20 minutes.

11. Save the readings after the kinetic protocols are finished. The exchange rate can be calculated by reducing the data to max slope (using 12 pts) or Vmax with the software that accompanies the plate reader. The exchange curve can be achieved by export to Microsoft Excel.

Figure 2. SOS1-ExD protein mediated Mant-GDP exchange on K-Ras4B in the presence of NSC658497.



Legend: Ras protein (2.5 µM) was pre-loaded with Mant-GDP using EDTA for exchange. The nucleotide was locked in place with excess Mg^{2+}. NSC658497 at different concentrations as shown was pipetted into wells of a black 384-well low volume plate. At time zero, 500 µM GTP was pipetted in to the wells and the reactions were monitored for 20 min by reading every 30 sec..

Product Uses

- Study inhibitors of SOS1 GTP exchange activity
- Identification of SOS1 binding proteins
- Study of SOS1 GEF activity with different GTPases.

Product Citations/Related Products

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