



# Datasheet

V. 1.0

## SOS1 exchange domain (564-1049) protein

(Human recombinant)

Cat. # CS-SOS1

Lot # 011

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

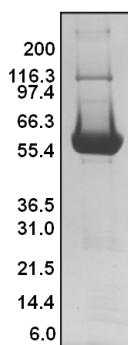
The protein should be reconstituted to 50 µM (3.03 mg/ml) by the addition of 33 µl of distilled water. The protein will be in the following buffer: 17 mM Tris pH 7.5, 17 mM NaCl, 0.2 mM MgCl<sub>2</sub>, 2.3% sucrose, 0.3% dextran. In order to maintain high biological activity of the protein, it is strongly 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

Human SOS1 is an exchange factor for the small G-protein Ras. The biological activity of SOS1 can be determined from its ability to catalyze the exchange of GDP for GTP on Ras. A standard biological assay for monitoring the catalytic activity of hSOS1 is an exchange assay utilizing the 2x Exchange Buffer from the Rho-GEF exchange assay kit (Cat.# BK100).

### Reagents

1. Recombinant K-Ras protein
2. Recombinant SOS1-ExD protein (Cat.# CS-SOS1)
3. 2x Exchange Buffer (40 mM Tris pH 7.5, 100 mM NaCl, 20 mM MgCl<sub>2</sub> , 0.1 mg/ml BSA, 1.5 µM mant-GTP)
4. Dilution Buffer (20 mM Tris pH 7.5, 50 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA)

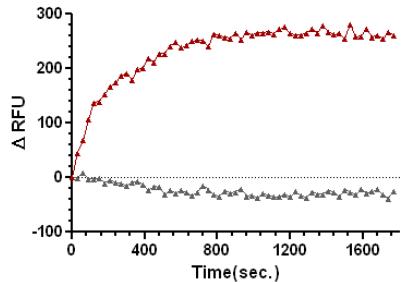
### Equipment

1. Fluorescence spectrometer ( $\lambda_{ex}=360\text{nm}$ ,  $\lambda_{em}=440\text{nm}$ )
2. Corning 96-well half area plates (Cat # 3686) or other plate with low protein binding surface.

### Method

1. Dilute SOS1-ExD protein (Cat# CS-SOS1) to 6 µM (0.36 mg/ml) with Dilution Buffer.
2. Dilute K-Ras to 50 µM (1.1 mg/ml) with Dilution Buffer.
3. Dissolve lyophilized 2x Exchange Buffer in 5 ml milli-Q water and keep in room temperature.
5. Set up the plate reader for kinetic fluorescence measurements (Excitation wavelength at 360 nm and emission wavelength at 440 nm) with readings every 30 seconds for 30 minutes.
6. Add the following components together and mix well by gentle pipeting:

	Exchange reaction mix	96 well black plate
2x Exchange Buffer		50 µl
dH <sub>2</sub> O		36 µl
50 µM K-Ras		4 µl
5.	Pipette 10 µl of 6 µM SOS1-ExD protein or Dilution Buffer in their respective wells and immediately pipette up and down twice and begin reading the fluorescence.	
6.	Once the readings are complete and the plate reader file has been saved, the exchange rate can be calculated by reducing the data to Vmax with the software that accompanies the plate reader.	



**Figure 2. SOS1-ExD protein mediated mant-GTP exchange on K-Ras.**

K-Ras protein was added to the wells of a 96-well half area plate containing diluted Exchange Buffer and mixed well. To initiate the exchange reaction, SOS1-ExD protein (red triangles), or Dilution Buffer (grey triangles), was added to the wells, mixed, and fluorescence measurements were obtained using a Tecan SpectraFluor Plus Spectrophotometer. The average fluorescence data from duplicate assays were normalized to the fluorescence at the zero time point. The resulting data were plotted as the change in relative fluorescence units ( $\Delta$ RFU) over time using GraphPad Prism software.

#### 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

For the latest citations and related products please visit [www.cytoskeleton.com](http://www.cytoskeleton.com).