V. 1.0

K-Ras4B Protein: K128A
(Human recombinant, 6xHis-tag)
Cat. # CS-RS08
Lot: 011 Amount: 1 x 100 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material

The K128A (lysine to alanine at amino acid position 128) mutant human K-Ras4B protein has been produced in a bacterial expression system. The recombinant protein contains six histidine residues at its amino terminus (His-tag). The molecular weight of 6xHis tagged K128A K-Ras4B is approximately 25 kDa and it is supplied as a white lyophilized powder. Note: The K128A mutation endows the protein with endogenous exchange activity even in the presence of Mg^{2+} ions (see Figure 2).

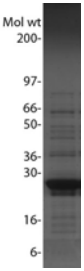
Storage and Reconstitution

Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml with the addition of 20 µl of Milli-Q water (100 µg size). When reconstituted, the protein will be in the following buffer: 50 mM Tris pH 7.5, 50 mM NaCl, 0.5 mM $MgCl_2$, 5% (w/v) sucrose, and 1% (w/v) dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution be supplemented with DTT to 1 mM final concentration, aliquoted into "experiment-sized" amounts, snap frozen in liquid nitrogen, and stored at -70°C. The protein is stable for six months if stored at -70°C. **The protein should not be exposed to repeated freeze-thaw cycles.** The lyophilized protein is stable at 4°C desiccated (<10% humidity) for one year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue-stained protein on a 4-20% polyacrylamide gradient gel. His tagged K128A K-Ras4B protein was determined to be >90% pure. (see Figure 1).

Figure 1. K128A K-Ras4B Protein Purity Determination.



A 20 µg sample of recombinant K128A K-Ras4B protein (molecular weight approx. 25 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). SeeBlue molecular weight markers are from Life Technologies Inc.

Biological Activity Assay

The biological activity of K128A K-Ras4B can be determined from the ability of the SOS1 exchange domain (SOS1-ExD) to catalyze the exchange of GDP for GTP onto mutant K-Ras4B. A standard biological assay for monitoring the biological activity of K128A K-Ras4B is an exchange assay utilizing the 2X Exchange Buffer from the RhoGEF exchange assay kit (Cat.# BK100). The Exchange Buffer contains Mant-GTP as reporter, which can be changed for Bodipy-GDP or -GTP for 490/513nm range and greater sensitivity.

Reagents

1. Recombinant K128A K-Ras4B protein (Cat.# CS-RS08)
2. 2X Exchange Buffer (40 mM Tris pH 7.5, 100 mM NaCl, 20 mM $MgCl_2$, 0.1 mg/ml BSA, 1.5 µM mant-GTP)
3. Dilution Buffer (20 mM Tris pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 0.1 mg/ml BSA)

Equipment

1. Fluorescence spectrophotometer (λex=360 nm, λem=440 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 1 µM (0.06 mg/ml) with Dilution Buffer.
2. Dilute K128A K-Ras4B to 40 µM (1.0 mg/ml) with Dilution Buffer.
3. Dissolve lyophilized 2X Exchange Buffer in 5 ml nanopure water and keep at 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 pipetting:

Exchange reaction mix	96 well black plate
2X Exchange Buffer	50 µl
dH_2O	26 µl
40 µM K128A K-Ras4B	4 µl

5. Pipette 20 µl of 1 µM SOS1-ExD protein or Dilution Buffer into 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.

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Product Uses

- Drug binding studies with the compounds that bind in the groove occupied by K128.
- Study of K128A K-Ras4B exchange activity with different GEFs
- Identification of K128A K-Ras4B exchange factors (GEFs)
- Positive control for GEF studies
- Biochemical characterization of K128A K-Ras4B protein interactions
- Western blot standard

Key References for K-Ras4B mutations

Ostrem J. et. al. 2013. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature*. 503, 548-551.

Prior I.A. et al. 2012. A comprehensive survey of Ras mutations in cancer. Cancer Res. 72(10): p.2457–2467. doi:10.1158/0008-5472.CAN-11-2612.

Welsch M.E. et al. 2017. Multivalent small-molecule pan-Ras inhibitors. Cell 168, 878–889. doi: 10.1016/j.cell.2017.02.006.

Product Citations/Related Products

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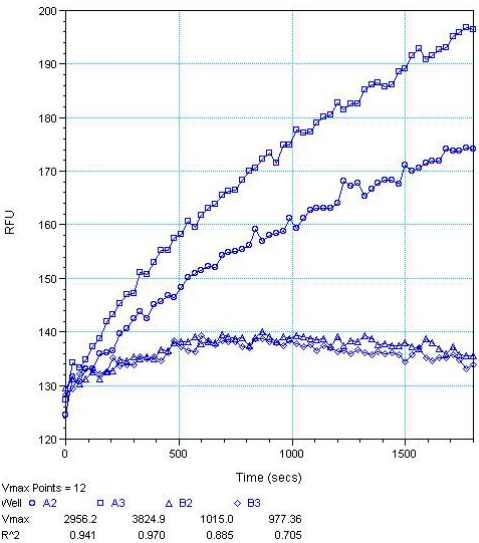
Figure 2. GTP-exchange assay with KRas4B K128A.



Legend: KRas4B K128A (Cat. # CS-RS08) was used in a nucleotide exchange reaction. Bodipy-GTP from ThermoFisher was used as a reporter at 1 µM and KRas was at 2 µM. Ex/Em was 490/513nm. Well A2 = K-Ras4B K128A without EDTA. A3 = K-Ras4B K128A with 10 mM EDTA, B2 Buffer only, and B3 = Buffer with 10 mM EDTA. Note: The K128A mutation endows the protein with endogenous exchange activity even in the presence of Mg^{2+} ions (A2).