V. 1.0

KIF7 Motor Domain: amino acids 1-370
(human recombinant)
Cat. # CS-KF51

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

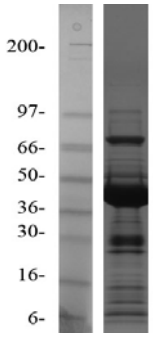
Material

The conserved motor domain of human kinesin KIF7 was expressed in *E.coli*. The recombinant protein contains a 6 histidine tag at the amino terminal end and has a combined molecular weight of 44 kDa. The protein has been determined to be biologically active in a microtubule-activated ATPase/GTPase activity test (see below). The protein is supplied as a lyophilized powder.

EG01size	Minimum amt. per tube	Actual amt. per tube	Vol. of buffer for 5 mg/ml resuspension	ATPase (nmol/min/mg)	GTP ATPase (nmol/min/mg)
KF51-A,B	25 µg	25 µg	5 µl	40	230
KF51-XL	N.A.				

Storage and Reconstitution

The lyophilized protein is stable for 1 year when stored at 4°C with a desiccant (humidity <10%). Alternatively, the lyophilized protein can be stored at -70°C and is stable for 1 year. The protein should be reconstituted to 5 mg/ml with distilled water or CMW Buffer 1 (100 mM PIPES pH 7.0, 200 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 20 µM ATP). See the table above for resuspension volumes. The protein can be aliquoted into experiment sized aliquots, snap frozen in liquid nitrogen and stored at -70°C. Under these conditions the protein is stable for 4 months. For working concentrations the Kif7 protein should be diluted in CMW Buffer 1. NOTE: kinesins do not respond well to repeated freeze/thaws and for storage at -70°C the protein concentration should not be less than 5 mg/ml. Kinesin diluted below 5 mg/ml should not be re-frozen as it will lose its biological activity.



Purity

Protein purity is determined by scanning densitometry of a Coomassie-stained SDS-PAGE gradient gel. Figure 1 shows 20 µg of KF51 protein and purity was determined to be >80%. The total protein in each tube will therefore be approximately 20% greater than the amount shown on the tube.

Figure 1. Kif7 Motor Domain Purity Gel. A 20 ug sample of recombinant Kif7 motor domain protein (His6-tagged) was separated on a 4-20% SDS-PAGE gradient gel, along with Mark12 molecular weight markers (Life Tech.). The recombinant Kif7 protein has an apparent molecular weight of 44 kDa. Protein quantitation was determined using Advanced Protein assay (Cat. # ADV02).

Microtubule Activated ATPase ASSAY

KIF7 ATPase and GTPase activities were measured by monitoring real time free phosphate generation using the Kinesin ELIPA Assay Kit (Cat. # BK060). The assay is based upon an absorbance shift (330 nm to 360 nm) that occurs when 2-amino-6-mercapto-7-methylpurine ribonucleoside (MESG) is catalytically converted to 2-amino-6-mercapto-7-methylpurine in the presence of inorganic phosphate (Pi). One molecule of Pi will yield one molecule of 2-amino-6-mercapto-7-methylpurine in an essentially irreversible reaction. Hence, the absorbance at 360 nm is directly proportional to the amount of Pi generated in the kinesin ATPase/GTPase reaction. Under the conditions outlined below, the Vmax for Kif7 microtubule-activated ATPase activity was 40 nmoles Pi generated per minute per mg of Kif7 and for the GTPase activity it was 230 nmoles Pi generated per min per mg (Figure 2).

Reagents

1. Kinesin ELIPA Assay Kit (Cat. # BK060)

Equipment

Monochromatic spectrophotometer (set to 360 nm) or a filter based spectrophotometer with a 360 nm filter and bandwidth of <10 nm.

Method (ELIPA ATPase assay)

The reactions were conducted in 96 well plates (300 ul reaction volumes). Each reaction contains 5 ug of Kif7 protein (EG01), 0.7 µM taxol stabilized microtubules (Cat. # MT001), 0.2 mM MESG, 0.3U PNP (purine nucleotide phosphorylase), 15 µM taxol, 15 mM PIPES pH 7.0, 5 mM $MgCl_2$, 0.6 mM ATP. Control reactions were carried out in the absence of KF51. These reactions gave readings of <0.1 OD over 20 min. Reactions were measured in a SpectraMax 250 (Molecular Devices) set in kinetic mode and 360nm absorbance wavelength. Readings were taken at room temperature once every 30 seconds for a total reaction time of 20 minutes. Typical assay results are shown in Figure 2. The nmoles of Pi generated in a given time was determined by the use of a phosphate standard curve (not shown).

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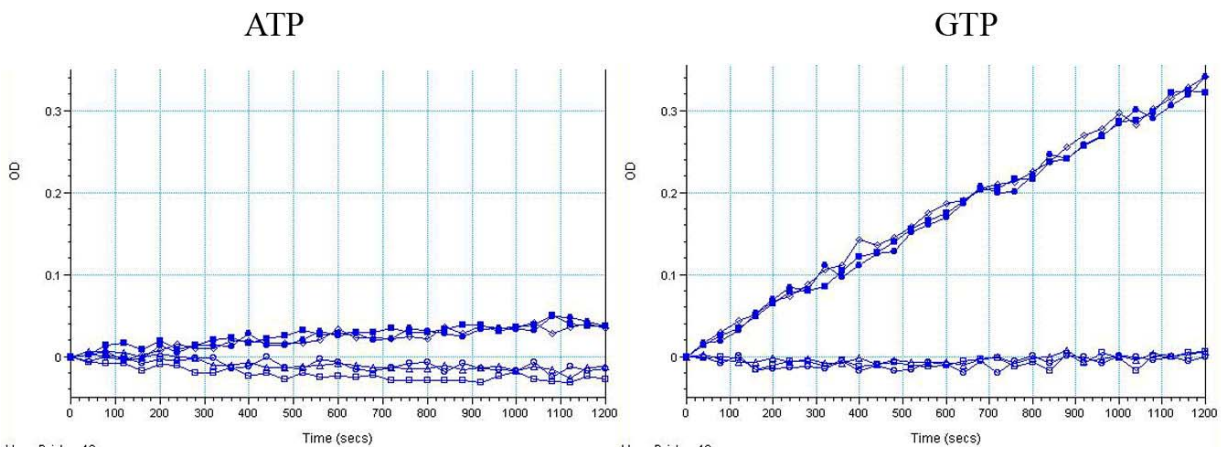

Figure 2. KF51 microtubule-activated ATPase/GTPase activity using the Kinesin ELIPA Assay Kit (Cat. # BK060). Kif7 ATP or GTPase activity was assayed in duplicate according to the method described. Control reactions without microtubules (open symbols) were compared to those with microtubules (closed symbols).

Product Uses

- Measurement of microtubule-activated ATPase/GTPase assays
- Identification/characterization of proteins or small molecules that affect motor ATPase/GTPase activity
- Identification/characterization of proteins or small molecules that affect motor/microtubule interactions.

References

1. Funk, CJ. et al., 2004. *Anal Biochem*. 329:68-76.
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3. Lockhart, A and Cross, RA. 1996. *Biochemistry*. 35:2365-2373
4. Sawin, KE. et al. 1992. *Nature*. 359:540-543.

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