

Hs KIFC3 Motor Domain

Cat. # KC01

Upon arrival store at 4°C (desiccated)

See datasheet for storage after reconstitution

Material

The conserved motor domain of human KIFC3 was expressed in a prokaryotic system. The recombinant protein contains a GST-Tag at the amino terminal end and has a combined molecular weight of 75 kD. The protein has been determined to be biologically active in a microtubule-activated ATPase activity test (see below). The protein is supplied as a lyophilized powder.

KC01 Size	Minimum amt. per tube	Actual amt. per tube	Vol. of buffer for 5 mg/ml resuspension	Minimum* ATPase (V _{max}) (nmol/min/mg)	Minimum* ATPase (Endpoint) (nmol/min/mg)
KC01-A,B	25 µg	25 µg	5 µg	80	50
KC01-XL	N.A.**	N.A.	N.A.		

*Guaranteed minimum ATPase activity. For actual activity of this Lot, see p. 3.

**Not available in this Lot. Ask Customer Service for availability of KC01-XL.

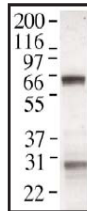
Storage and Reconstitution

The lyophilized protein is stable for at least 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 at least 1 year. The protein should be reconstituted to 5 mg/ml with distilled water or CMW Buffer 1 (100 mM PIPES pH 7, 200 mM KCl, 2 mM MgCl₂, 1 mM DTT, 20 µM ATP). See above table for resuspension volumes. The protein can be aliquoted into experiment sized tubes and snap frozen in liquid nitrogen. When reconstituted and stored at -70°C, the protein will be stable for at least 4 months. For working concentrations the KifC3 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 activity.

Purity

Protein purity is estimated by scanning densitometry of a coomassie-stained SDS-PAGE gradient gel. Figure 1 shows 10 µg of KC01 protein and purity was determined to be >60%. The total protein in each tube will therefore be approximately 40% greater than the amount shown on the tube. The major contaminant at approximately 30 kD is GST protein. The microtubule-activated ATPase activity of the KIFC3 motor is not inhibited by this contaminant.

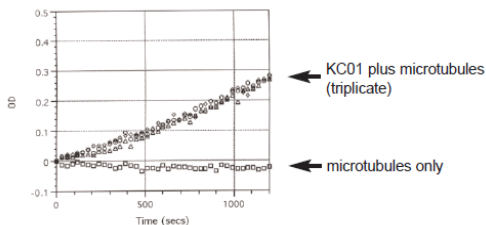
Figure 1. KifC3 Motor Domain protein gel. A 10 µg sample of recombinant KifC3 Motor Domain protein (GST-tagged) was separated on a 4-20% SDS-PAGE gradient gel, along with Mark12 molecular weight markers (Invitrogen). The fusion protein runs at 65 kD on the polyacrylamide gradient gel. Protein quantitation was determined using Advanced Protein assay (cat.# ADV01).



MICROTUBULE ACTIVATED ATPase ASSAY

KifC3 ATPase activity was 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 - 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 reaction. Under the conditions outlined below, the V_{max} for KifC3 microtubule-activated ATPase activity for this Lot was 90 nmoles ATP generated per minute per mg of KC01 (Figure 2). The ATPase rate for this Lot using a 10 minute endpoint assay (Kinesin ATPase End Point Assay Kit, cat.# BK053) was 75 nmoles ATP per minute per mg of KC01. Both of these values are above the guaranteed minimum.

Figure 2. KC01 microtubule-activated ATPase activity using the Kinesin ELIPA Assay Kit (cat.# BK060).



Reagents

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

Equipment

1. 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 μ l reaction volumes). Each reaction contains 10 μ g of KifC3 protein (KC01), 0.7 μ M taxol stabilized Microtubules (cat# MT001), 0.2 mM MESG, 0.3U PNP, 15 μ M taxol, 15 mM PIPES pH 7, 5 mM $MgCl_2$, 0.6 mM ATP. Control reactions were carried out in the absence of KC01. These reactions gave readings of <0.1. 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. The nmoles of ATP generated in a given time was determined by the use of a phosphate standard curve (not shown).

Product Uses

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

References

1. Hackney, D and Jiang, W. (2001) *Methods in Molecular Biology* (Humana Press) 164:65-71
2. Hoang, EH et al. 1998. *Genomics* 52:219-222.
3. Noda, Y et al. 2001. *J. Cell Biol.* 155:77-88.

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