V. 1.0

BimC Motor Domain (*Aspergillus fumigatus*)
(His-tagged)
Cat. # EG02

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

Material

The conserved motor domain of *Aspergillus fumigatus* BimC kinesin has been produced and purified from a prokaryotic expression system. The recombinant protein contains six histidine residues at the amino terminus (His-tag)and has an approximate molecular weight of 50 kDa. *A. fumigatus* BimC has been determined to be biologically active in a microtubule-activated ATPase activity test (see below). The protein is supplied as a white lyophilized powder.

EG02size	Minimum amt. per tube	Actual amt. per tube	Vol. of buffer for 5 mg/ml resuspension	Minimum* ATPase (Vmax) (nmol/ min/mg)	Minimum* ATPase (Endpoint) (nmol/min/mg)
EG02-A,B	15 µg	15 µg	3 µl	250	350
EG02-XL	1.0 mg	1.0 mg	200 µl	250	350

Storage and Reconstitution

The lyophilized protein is stable for 1 year when stored desiccated at 4°C (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 Milli-Q water and will be in the following buffer: 100 mM PIPES pH 7.0, 300 mM KCl, 2 mM $MgCl_2$, 1 mM DTT and 30 µM ATP). The protein can be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at -70°C. Under these conditions the protein is stable for 4 months. For working concentrations, the BimC protein should be diluted in CMW Buffer 1 (100 mM PIPES pH 7.0, 300 mM KCl, 2 mM $MgCl_2$, 1 mM DTT and 30 µM ATP). NOTE: Kinesin proteins 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 Coomassie stained protein on a 12% gel. His-BimC protein was determined to be >95% pure (see Figure 1).

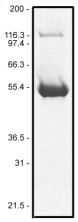

Figure 1. His-BimC Kinesin Motor Domain protein purity gel. A 10 µg sample of recombinant His-BimC protein (approx. 50 kDa) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie blue. Protein quantitation was determined using Advanced Protein assay (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

MICROTUBULE ACTIVATED ATPase ASSAY

BimC 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 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 reaction. Under the conditions outlined below, the Vmax for BimC microtubule-activated ATPase activity has a minimum activity of 250 nmoles ATP generated per minute per mg of protein (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 µl reaction volumes). Each reaction contains either 2 µg of BimC protein (Cat. # EG02) or 2 µg of Eg5 protein (Cat. # EG01) and 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 protein. 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 360 nm absorbance wavelength. Readings were taken at room temperature once every 30 s for a total reaction time of 20 min. Typical assay results are shown in Figure 2. The nmoles of ATP generated in a given time was determined by the use of a phosphate standard curve (not shown).

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

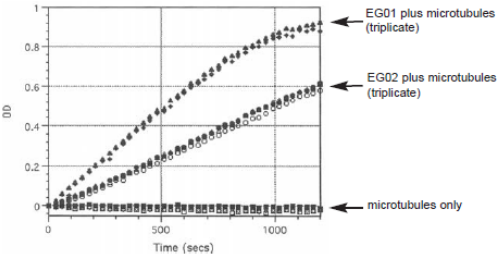

Figure 2. EG02 microtubule-activated ATPase activity. Recombinant BimC from *A. fumigatus* was assayed for microtubule-activated ATPase activity in triplicate along with human recombinant Eg5 (Cat. # EG01) according to the method described. Control reactions were carried out in the absence of motor protein (microtubules only) and in the absence of microtubules (data 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. Funk, CJ. et al., 2004. *Anal Biochem*. 329:68-76.
2. Hackney, D and Jiang, W. 2001. *Methods in Molecular Biology* (Humana Press). 164:65-71
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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