V. 2.0

Hs Eg5 Motor Domain
(human recombinant)
Cat. # EG01

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

Material
The conserved motor domain of human kinesin Eg5 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 74 kDa. 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.

EG01 size	Minimum amt. per tube	Minimum ATPase (Vmax) (nmol/min/mg)
EG01-A,B	25 µg	600
EG01-XL	1 mg	600

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). The protein can be aliquoted into experiment sized tubes, snap frozen in liquid nitrogen and stored at -70°C. Under these conditions the protein is stable for 4 months. For working concentrations the Eg5 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.

Reference
Funk CJ, Davis AS, Hopkins JA, Middleton KM. 2004. Development of high-throughput screens for discovery of kinesin adenosine triphosphatase modulators. Anal Biochem. 2004 329:68-76.

Purity
Protein purity is estimated by scanning densitometry of a Coomassie Blue stained SDS-PAGE gradient gel. Figure 1 shows 10 µg of EG01 protein and purity was determined to be >90%. The major contaminant at approximately 30 kDa is GST protein. The microtubule-activated ATPase activity of the Eg5 motor is not inhibited by this contaminant.

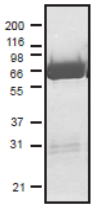

Figure 1. Eg5 Motor Domain protein gel. A 10 µg sample of recombinant Eg5 motor domain protein (GST-tagged) was separated on a 4-20% SDS-PAGE gradient gel, along with Mark12 molecular weight markers (Invitrogen). The fusion protein runs at 74 kDa on the polyacrylamide gel. Protein quantitation was determined using Advanced Protein assay (Cat. # ADV02).

MICROTUBULE ACTIVATED ATPase ASSAY
Eg5 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 Eg5 microtubule-activated ATPase activity is >600 nmoles ATP generated per minute per mg of EG01 (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 5 µg of Eg5 protein (EG01), 0.7 µM taxol stabilized microtubules (Cat. # MT002), 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 EG01. 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 ATP generated in a given time was determined by the use of a phosphate standard curve (not shown).

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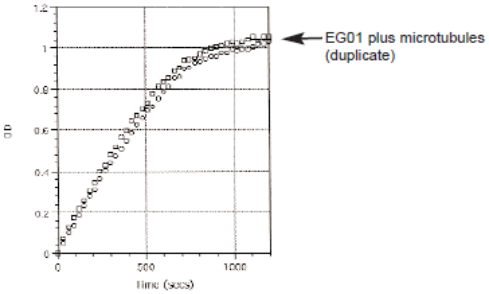

Figure 2. EG01 microtubule-activated ATPase activity using the Kinesin ELIPA Assay Kit (Cat. # BK060). Eg5 ATPase activity was assayed in duplicate according to the method described. Control reactions were carried out in the absence of motor protein and in the absence of microtubules, both control reactions gave negligible ATPase activity (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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