V. 1.2

Hs CENP-E Motor Domain

Cat. # CP01

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

Material

The conserved motor domain of human CENP-E 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 71 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 white lyophilized powder.

CP01 size	Minimum amt. per tube	Minimum* ATPase (Vmax) (nmol/min/mg)	Minimum* ATPase (Endpoint) (nmol/min/mg)
CP01-A,B	25 µg	900	900
CP01-XL	1 mg	900	900

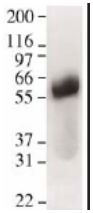
Storage and Reconstitution

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 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 $MgCl_2$, 1 mM DTT, 20 µM ATP). 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 4 months. For working concentrations the CENP-E 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 refrozen as it will lose activity.

Purity

Protein purity is estimated by scanning densitometry of a Coomassie Blue stained SDS-PAGE gradient gel. Figure 1 shows 10 µg of CP01 protein and purity was determined to be >90%. The total protein in each tube will therefore be approximately 10% greater than the amount shown on the tube. The major contaminant at approximately 30 kDa is GST protein. The microtubule-activated ATPase activity of the CENP-E motor is not inhibited by this contaminant.

Figure 1. CENP-E Motor Domain protein gel

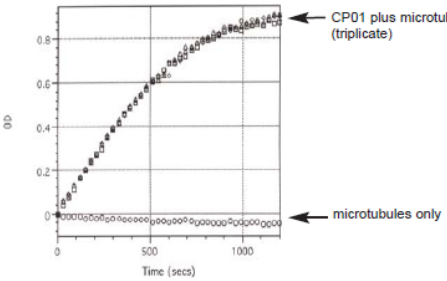


A 10 µg sample of recombinant CENP-E Motor Domain protein (GST-tagged) was separated on a 4-20% SDS-PAGE gradient gel, along with Mark12 molecular weight markers (Invitrogen). The fusion protein runs at 65 kDa on the polyacrylamide gradient gel. Protein quantitation was determined using Advanced Protein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

Microtubule Activated ATPase Assay

CENP-E 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 Vmax for CENP-E microtubule-activated ATPase activity is >900 nmoles ATP generated per minute per mg of CP01 (Figure 2). The ATPase rate using a 10 minute endpoint assay (Kinesin ATPase End Point Assay Kit, Cat. # BK053) is >900 nmoles ATP per minute per mg of CP01 (data not shown).

Figure 2. CENP-E 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 µl reaction volumes). Each reaction contains 2 µg of CENP-E protein (Cat. # CP01), 0.7 µM taxol stabilized Microtubules (Cat. # MT001), 0.2 mM MESG, 0.3U PNP, 15 µM taxol, 15 mM PIPES pH 7, 5 mM $MgCl_2$, 0.6 mM ATP. Control reactions were carried out in the absence of CP01. These reactions gave readings of <0.1. Reactions were measured in a SpectraMax250 (Molecular Devices) set

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in kinetic mode and 360 nm 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. Wood, KW et al. (1997) *Cell* 91:357-366.
3. Yen et al. (1991) *EMBO Journal* 10:1245-1254.

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