V.2.0

Kinesin Heavy Chain Motor Domain
(*H.sapiens* recombinant)
Cat. # KR01

Upon arrival store at 4°C (dessicated) or at –70°C
See datasheet for storage after reconstitution

Material

The conserved motor domain of human Kinesin Heavy Chain has been produced in a bacterial expression system. The recombinant protein contains a GST-tag at the amino terminus and has a combined calculated molecular weight of approximately 70 kDa. The protein has been determined to be biologically active in a microtubule-activated ATPase assay (see below). The protein is supplied as a white lyophilized powder.

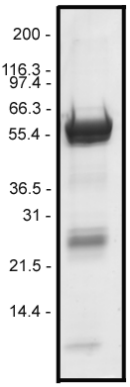
Table 1: KR01 information

KR01 size	Minimum amt. per tube	Vol. of buffer for 5 mg/ml resuspension	Minimum* ATPase (Vmax) (nmol/min/mg)
KR01-A,B	25 µg	5 µl	3500
KR01-XL	1 mg	200 µl	3500

*Guaranteed minimum ATPase activity.

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on an SDS gradient gel. Figure 1 shows 10 µg of KR01 protein that was determined to be 88% pure. The major contaminant at approximately 30 kDa is GST protein. The microtubule-activated ATPase activity of the Kinesin Heavy Chain is not inhibited by this contaminant.

Figure 1. Kinesin Heavy Chain Motor Protein Purity Determination. A 10 µg sample of recombinant Kinesin Heavy Chain Motor Domain protein was separated on a 4-20% SDS gel along with Mark12 molecular weight markers (Invitrogen) and stained with Coomassie Blue. The fusion protein has an approximate molecular weight of 65 kDa. Protein quantitation was determined using the Precision Red™ Protein Assay reagent. (Cat. # ADV02).



Storage and Reconstitution

Shipped at ambient temperature. The recommended storage conditions for the lyophilized material is 4°C and <10% humidity. Under these conditions the protein is stable for 1 year. Alternatively the lyophilized protein can be stored at –70°C and is stable for 1 year.

Briefly centrifuge to collect the protein at the bottom of the tube. 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, and 20 µM ATP). See Table 1 for resuspension volumes. The protein can be aliquoted into experiment sized amounts 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 Kinesin Heavy Chain protein should be diluted in CMW Buffer 1. **NOTE: Kinesins do not respond well to repeated freeze/thaw cycles and for storage at –70°C the protein concentration should not be less than 2-5 mg/ml. Kinesin diluted below 2 mg/ml should not be re-frozen as it will lose activity**.

Microtubule Activated ATPase Assay

Kinesin Heavy Chain ATPase activity was measured by monitoring real time free phosphate generation using the Kinesin ELIPA Assay Biochem 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.

Reagents

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

Equipment

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

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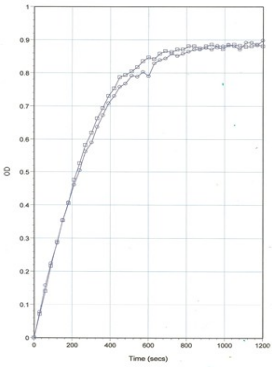
Method (ELIPA ATPase Assay)

The reactions were conducted in a 96 well plate (300 µl reaction volumes). Each reaction contains 2 µg of Kinesin Heavy Chain protein (Cat. # KR01), 0.7 µM taxol stabilized Microtubules (Cat.# MT002), 0.2 mM MESG, 0.3U PNP, 15 µM taxol, 15 mM PIPES pH 7.0, 5 mM $MgCl_2$ and 0.6 mM ATP. Control reactions were carried out in the absence of KR01. These reactions gave readings of < 0.1 (not shown). Reactions were measured in a SpectraMax 250 (Molecular Devices) set in kinetic mode at 360 nm absorbance wavelength. Readings were taken at room temperature once every 30 s for a total reaction time of 20 min. The nmoles of ATP generated in a given time was determined by the use of a phosphate standard curve (not shown).

Figure 2. ***KR01 Microtubule Activated ATPase Activity using Kinesin ELIPA Assay Biochem Kit***



Legend: Duplicate reactions containing KR01 and microtubules were performed as described using the Kinesin ELIPA Assay Biochem Kit (Cat. # BK060)

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.
- Microtubule motility assays.

References

1. Hackney, D and Jiang W. 2001. Assays for Kinesin Microtubule-Stimulated ATPase Activity. *Methods in Molecular Biology* (Humana Press) **164**, 65-71.

2. Ma, YZ and Taylor, EW. 1995. Mechanism of Microtubule Kinesin ATPase. *Biochemistry.* **34**, 13233-13241.

3. Navone, F. et al. 1992. Cloning and expression of a human kinesin heavy chain gene: interaction of the COOH-terminal domain with cytoplasmic microtubules in transfected CV-1 cells. *J. Cell. Biol.* **117**, 1263-1275.

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