V. 1.1

KIF22 Motor Domain (5-378) His-Protein: wild-type (Human recombinant)
Cat. # CS-KF02
Lot # 017 1 x 100 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material

The wild-type human motor domain of the kinesin-like KIF22 protein (also known as kinesin-like DNA binding protein, KID) has been produced in a bacterial expression system. The recombinant protein contains a 6x His-tag at the amino terminal-end and amino acids 5-378. The molecular weight of KIF22 is approximately 43 kDa and it is supplied as a white lyophilized powder.

Storage and Reconstitution

Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml with the addition of 20 µl of nanopure water (100 µg size). When reconstituted, the protein will be in the following buffer: 100 mM Tris pH 8.0, 100 mM NaCl, 2 mM $MgCl_2$, 15% (w/v) sucrose, and 1% (w/v) dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution be supplemented with DTT to 1 mM final concentration, aliquoted into "experiment-sized" amounts, snap frozen in liquid nitrogen, and stored at -70°C. The protein is stable for six months if stored at -70°C. Defrosting should be in a room temperature water bath for 3 min then place in ice. Dilution of protein must be done in 100 mM Tris pH 8.0 and 100 mM NaCl buffer. **The protein should not be exposed to repeated freeze-thaw cycles.** The lyophilized protein is stable at 4°C desiccated (<10% humidity) for one year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue-stained protein on a 4-20% polyacrylamide gradient gel. Kif22 5-378 protein was determined to be 90% pure. (see Figure 1).

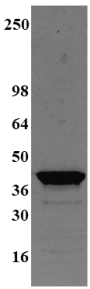

Figure 1. KIF22 5-378 Protein Purity Determination. A 10 µg sample of recombinant KIF22 protein (molecular weight approx. 43 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). Seeblue Pre-stained molecular weight markers are from Invitrogen.

Biological Activity

Microtubule activated ATPase Assay

KIF22 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 KIF22 microtubule-activated ATPase activity is >1200 nmoles ATP generated per minute per mg of KIF22 (Figure 2).

Reagents

1. Kinesin ELIPA Assay Kit (Cat.# BK060)
2. Recombinant KIF22 protein (Cat.# CS-KF02)

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)

1. Thaw out and aliquot ELIPA reaction buffer, ELIPA 1 and 2 reagents, microtubules, taxol, and ATP reagents as described in the BK060 datasheet (https://www.cytoskeleton.com/bk060).
2. Resuspend 1-vial of KIF22 to 1 mg/ml with 100 µl 100 mM Tris-HCl pH=8.0 and 100 mM NaCl buffer.
3. Mix the components together **IN THE ORDER SHOWN IN TABLE 1 BELOW**;

Table 1. MT ELIPA MIX

Order	Reagent	Volume
1	ELIPA Reaction Buffer (room temperature)	2 ml
2	Taxol stock	20 µl
3	MT's	160 µl
4	ELIPA Reagent 1	480 µl
5	ELIPA Reagent 2	24 µl

4. Gently swirl MT ELIPA MIX to mix evenly and incubate for for 5 minutes at room temperature.
5. The MT ELIPA MIX is now ready for the addition of your motor protein.

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6. Aliquot 1 ml of MT ELIPA Mix to a 1.5 ml Eppendorf tube. This will be the no motor control.
7. Aliquot 1.5 ml of MT ELIPA Mix to 1.5 ml Eppendorf tube. Add 2 µl 1 mg/ml KIF22 to 1.5 ml MT ELIPA Mix and mix.
8. Aliquot 300 µl of no motor control to well positions A1-C1 in a 96-well plate.
9. Aliquot 300 µl of KIF22 MT ELIPA mix to well positions D1-G1 in a 96-well plate.
10. Setup the spectrophotometer in kinetic mode to take readings every 30 seconds for 30 min at room temperature/
11. Insert plate into spectrophotometer and start kinetic run.
12. After 3 mins or when baseline stabilizes, interrupt run, and aliquot 20 µl of working stock 10 mM ATP into each well with a multichannel pipettor to begin reactions simultaneously.
13. Immediately read the reactions again at 360 nm.

Figure 2. KIF22 microtubule ATPase activity determined with Kinesin ELIPA Kit (Cat#. BK060).

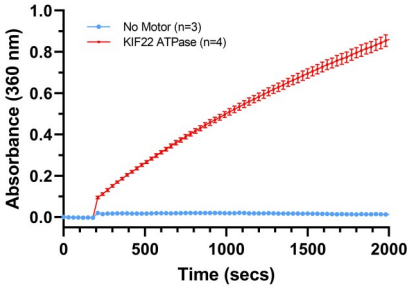

Figure legend: KIF22 (0.4 µg) ATPase activity was measured (n=4) at an absorbance of 360 nm on an iD5 multi-mode microplate reader (Molecular devices) over 30 minutes at room temperature. The ATPase activity was started when 0.6 mM of ATP was added 3 minutes into the kinetic run. Control reactions (n=3) were carried out in the absence of KIF22 motor protein.

Microtubule Activated Endpoint ATPase Assay

KIF22 ATPase activity was determined by measuring the inorganic phosphate (Pi) generated during the microtubule activated ATPase activity with the HTS Kinesin ATPase Endpoint Assay Biochem Kit (Cat.# BK053). This is a fast and economical method suitable for HTS applications. The assay is based on up a colorimetric change, measure at 650 nm.

Reagents

1. HTS Kinesin ATPase Endpoint Assay Biochem Kit (Cat.# BK053)
2. Recombinant KIF22 protein (Cat.# CS-KF02)

Method

1. Thaw out and aliquot kinesin reaction buffer, microtubules, taxol, kinesin motor protein, and ATP reagents as described in the BK053 datasheet (https://www.cytoskeleton.com/bk053).
2. Resuspend 1-vial of KIF22 to 0.5 mg/ml with 100 µl 100 mM Tris-HCl pH=8.0 and 100 mM NaCl buffer.
3. Transfer 240 µl 0.5 mg/ml KIF22 5-378 to a new 1.5 ml centrifuge tube with 510 ml 100 mM Tris-HCl pH=8.0 and 100 mM NaCl buffer to produce a 0.16 mg/ml KIF22 working stock.
4. Aliquot reagents as described in Table 2.

Table 2. MT Activated ATPase Reactions.

Well Position	Kinesin Reaction Buffer + Taxol	MTs (0.2mg/ml)	KHC Control Protein	KIF22 (0.16 mg/ml)	Well Designation
A1	30 µl	0	0	0	Blank
B1	20 µl	10 µl	0	0	MT only
C1	27.5 µl	0	2.5 µl	0	KHC only
D1	27.5 µl	0	0	2.5 µl	KIF22 only
E1	17.5 µl	10 µl	2.5 µl	0	KHC ATPase
F1	17.5 µl	10 µl	0	2.5 µl	KIF22 ATPase

5. Before adding the ATP, make sure spectrophotometer is set up correctly in end-point read mode at 650 nm.
6. To start the reactions, use a 8-well multichannel pipettor to aliquot 5 µl of ATP per reaction.
7. Allow the reactions to proceed at room temperature for 10 minutes.
8. Terminate reaction by adding 70 µl of CytoPhos to each well.
9. Allow the stop reaction to proceed for 10 minutes and take readings at 650 nm. Use well A1 (Table 2) as your blank.

Figure 3. KIF22 ATPase activity determined with HTS Kinesis ATPASE Endpoint Assay Biochem Kit (Cat#. BK053).

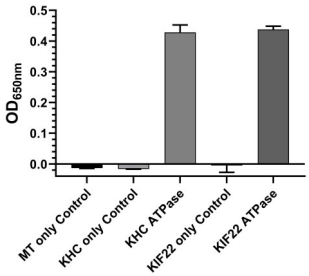

Figure legend: KIF22 (0.4 µg) ATPase activity was measured on a spectrophotometer at an absorbance of 650 nm in a 96-well half-area plate at room temperature.

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. Webb, M.R. 1992. A continuous spectrophotometric assay for inorganic phosphate and for measuring phosphate release kinetics in biological systems. Proc. Natl. Acad. Sci. USA 89: 4884-4887.
2. Kodama, T. et al. J. Biochem. 99: 1465-1472 (1986)
3. Walker, B.C. et al. Biochemistry 58: 2326-2338 (2019)

Product Citations/Related Products

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