KIF18B Motor Domain (1-365) His-Protein: wild-type (Human recombinant)
Cat. # CS-KF18B
Lot # 013 1 x 100 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

V. 1.0

Material
The wild-type human motor domain of the kinesin-like KIF18B protein has been produced in a bacterial expression system. The recombinant protein contains a 6x His-tag at the amino terminal-end and amino acids 1-365. The molecular weight of KIF18B is approximately 42 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 3 mg/ml with the addition of 33 µl of nanopure water (100 µg size). When reconstituted, the protein will be in the following buffer: 50 mM Tris-HCl pH 7.3 100 mM NaCl, 2 mM $MgCl_2$, 0.2% Triton X-100, 6% (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 50 mM Tris-HCl pH 7.3 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. KIF18B 1-365 protein was determined to be >85% pure. (see Figure 1).

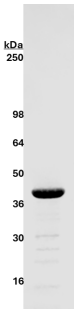

Figure 1. KIF18B 1-365 Protein Purity Determination. A 10 µg sample of recombinant KIF18B protein (molecular weight approx. 42 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity
Microtubule activated ATPase Assay
KIF18B 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 KIF18B micro-tubule-activated ATPase activity is >1500 nmoles ATP generated per minute per mg of KIF18B (Figure 2).

Reagents
1. Kinesin ELIPA Assay Kit (Cat.# BK060)
2. Recombinant KIF18B protein (Cat.# CS-KF18B)

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 KIF18B to 3 mg/ml with 33 µl nanopure water.
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
4	ELIPA Reagent 1	480 µl
3	MT's	160 µl
5	ELIPA Reagent 2	24 µl

4. Gently swirl MT ELIPA MIX to mix evenly and incubate for 5 minutes at room temperature.

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5. The MT ELIPA MIX is now ready for the addition of your motor protein.
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 3.3 µl of 3 mg/ml KIF18B to 1.5 ml MT ELIPA Mix and mix.
8. Aliquot 300 µl of no motor control to well positions A1-B1 in a 96-well plate.
9. Aliquot 300 µl of KIF18B MT ELIPA mix to well positions C1 -D1 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 27 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.

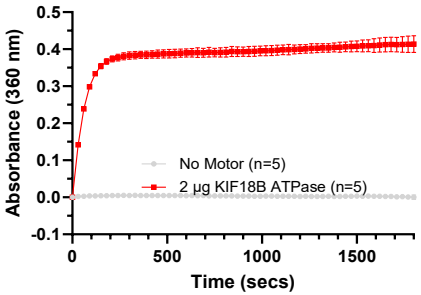

Figure 2. KIF18B microtubule ATPase activity determined with Kinesin ELIPA Kit (Cat#. BK060). Figure legend: KIF18B (2 µg) ATPase activity was measured (n=5) at an absorbance of 360 nm on an iD5 multi-mode microplate reader (Molecular devices) over 30 minutes at room temperature. The ATPase activity was started when 0.9 mM of ATP was added to the well before starting the kinetic run. Control reactions (n=5) were carried out in the absence of KIF18B motor protein.

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)

Product Citations/Related Products

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