Cytoskeleton, Inc.
The Protein Experts

Custom Services Newsletter

Oct 2013

This Issue contains

Future Topics

November 2013
Tubulin screening citations

December 2013
Molecular motors as clinical targets

January 2014
Myosin and the sarcomere

Catalog Products

Actin Proteins
Activation Assays
Antibodies
ECM Proteins
ELISA Kits
G-LISA® Kits
Pull-down Assays
Motor Proteins
Small G-Proteins
Tubulin & FtsZ Proteins

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A Snapshot of KIF7 in Disease

KIF7 is a Kinesin-4 family member that has been shown to play an important role in embryonic development and may play a role in the development of basal cell carcinomas. KIF7 functions in Hedgehog (Hh) signaling through the negative and positive regulation of the GLI family of transcription factors (i.e., GLI1, GLI2, and GLI3)[1]. While the mechanism of this regulation is still being elucidated, it appears that in the absence of an Hh ligand, KIF7 negatively regulates GLI transcription factor activity, and in the presence of Hh signaling it positively influences GLI dependent signaling; possibly through KIF7's Hh-dependent translocation from the base of primary cilia to the tip[1]. Mice lacking KIF7 exhibit a similar phenotype to mice lacking the GLI3 transcription factor, which is characterized by polydactyly (i.e., extra toes) and exencephaly (i.e., brain is located outside the skull)[2]. Loss-of-function mutations in the KIF7 gene in humans results in ciliopathies that can lead to lethal developmental abnormalities (e.g., Acrocallosal and fetal hydrolethalus syndrome) or less severe developmental defects (e.g., Meckel and Joubert syndromes)[3,4]. Given the important role of Hh signaling in the development of basal cell carcinomas (BCC) and other cancers[5], it will be interesting to see if research in this area also uncovers a role for KIF7 in Hh signaling-dependent cancers.

The Kif7 Motor Domain Exhibits Microtubule-Stimulated GTPase Activity

Cytoskeleton continues to expand their offerings of kinesin, dynein, and myosin motor assays with high signal to noise ratios. Recently, we have developed an assay for KIF7 (Kinesin-4 family) using the recombinantly expressed and purified KIF7(1-370) motor domain. This *E. coli*-expressed protein is >80% pure and when tested with our ATPase/GTPase Kinetic ELIPA Kit (Cat# BK051/52), we discovered a very strong microtubule (MT)-dependent GTPase activity (Fig. 1). This was a very unexpected finding given that kinesins are typically characterized as having MT-dependent ATPase activity. Consequently, these data either suggest a *bona fide* nucleotide preference of the KIF7 motor domain for GTP over ATP, or perhaps the GTPase activity is actually coming from tubulin in the MTs in a manner that is enhanced by KIF7. It is noteworthy that the kinesin heavy chain, Kinesin-1 family, is able to utilize GTP as well as ATP for its motor activity[6]. Additionally, other members of the Kinesin superfamily of motor proteins have been shown to modulate tubulin polymerization (Kinesin-7 and -10 family members)[7] or MT depolymerization (Kinesin-8 and -13 family members)[8], which are processes that are expected to affect the level of tubulin GTPase activity.

Cytoskeleton's Motor Werks Screening Services

At Cytoskeleton, we have developed assays and purified proteins for 11 recombinant Kinesin motor domains that represent 8 of the 14 recognized Kinesin families[9]. In addition, we have developed assays and proteins of cytoplasmic dynein and cardiac, skeletal, smooth, and non-muscle myosins, as well as an *in vitro* re-constituted cardiac sarcomere. These assays are available as a compound screening service with individual motors as well as multi-motor protein panel projects. If you need a different motor protein or assay, we offer custom protein expression/purification and assay development services to help move your project forward. For more information on available proteins and services, contact tservice@cytoskeleton.com for more information.

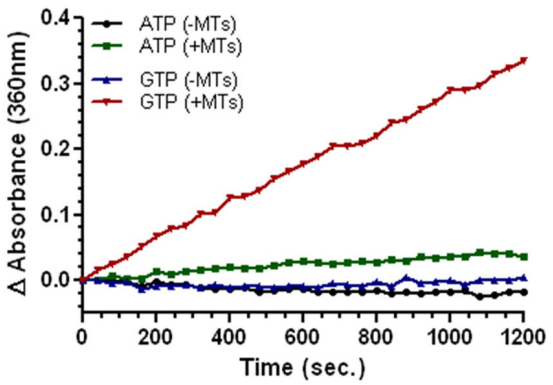

Figure 1. KIF7(1-370) Motor Domain Activity. The absorbance at 360 nm in the BK051/52 Kinetic ELIPA kit is directly proportional to the level of free phosphate in the assay that is generated by enzymatic hydrolysis of either ATP or GTP. MT = microtubules.

References

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7. Friel C.T. and Howard J. 2012. *J. Muscle Res. Cell. Motil*. **33**, 377-383.
8. Walczak C.E., Gayek S., and Ohi R. 2013. *Annu. Rev. Cell Dev. Biol*. **29**, 417-441.
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Custom Modules

Our recently expanded Custom Service department provides additional resources for your research projects.

Cytoskeleton is leading the way to develop novel kinesin, dynein, and myosin based compound screens.

We are scientists dedicated to providing accurate data reported in a detailed and timely manner.

About Custom Services

Like our regular product offerings, the Custom Service department emphasizes quality products and services. We understand that **accuracy** and **timeliness** are critical elements for a successful project. Choose from more than twenty defined modules (**for a full list, visit www.cytoskeleton.com/custom-services**), and then contact Technical Support (tservice@cytoskeleton.com) to guide you through the process.

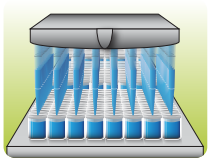
Clients Include:

- Merck & Co., Inc.
- Eli Lilly & Co.
- Amgen, Inc.
- Abbott Laboratories
- Pfizer, Inc.
- Astra-Zeneca plc
- GlaxoSmithKline plc
- Genentech, Inc.
- Johnson & Johnson
- Bristol-Meyers Squibb

Let's get started, it's as easy as 1,2,3 ...

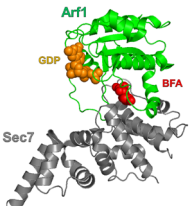
1. Choose a module and ask for a quote (24h turn around time)
2. Review quote, specifications, and deliverables
3. Place order and receive regular updates until project is finished

Compound Screening Modules



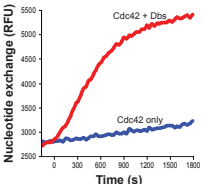
Type	Format	Deliverable	Module #	Timeline (wks)
Eg5 Kinesin motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	96 assays, consisting of 40 duplicate single concentrations (or 5 x IC50s), plus eight control wells. PDF Report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS050 or CDS051	2
Cardiac Myosin motor assay	Ca^{2+}/Sarcomere (thin filament) stimulated ATPase assay, kinetic, absorbance at 360nm	Same as CDS052.	CDS056	2
Dynein motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	Same as CDS052.	CDS065	2
Tubulin polymerization	Tubulin (>99% pure) Polymerization Assay, kinetic, fluorescence at 360nm/410nm	96 assays, with 40 duplicate single concentrations or 5 x IC50s, plus eight control wells (vinblastine, nocodazole or taxol). PDF Report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS009 or CDS010	2
GEF/GTPase exchange assay	GTP exchange factor plus Small G-protein (e.g. Rho or Ras) with mant-GTP reporter. Kinetic, fluorescence at 360nm/450nm	60 assays consisting of either 28 duplicate reactions plus 4 controls, or 5 x IC50s plus 1 x control IC50. PDF report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS100	2

Gene Cloning and Protein Purification Modules



Type	Name	Deliverable	Module #	Timeline (wks)
Recombinant Small Protein	Small protein or protein domain (<30 kDa) with gene provided by client	Highly purified, His-tagged active protein lyophilized in 10 x 100 µg aliquots (or more depending on yield). Datasheet and assay method. Activity in line with published articles. *E. coli* expression.	REC012	3
Recombinant Small Protein plus cloning	Small protein or protein domain (<30 kDa) including gene synthesis	Same as above with gene synthesis.	REC022	6
Recombinant Kinesin Motor-Protein	Medium to large protein or protein domain (30-100 kDa)	Same as REC012.	REC032	3
Recombinant Kinesin Motor Protein plus gene cloning	edium to large protein or protein domain (30-100 kDa) with gene synthesis	Same as above with gene synthesis.	REC042	8
Native or eukaryotic protein expression & purification	Cited protein purification	Same as above plus using a published procedure.	REC052	4-20

Assay Development Modules



Type	Name	Deliverable	Module #	Timeline (wks)
GTP Exchange (fluor. kinetic, 360nm/460nm)	G-protein GTP exchange assay using Mant-GTP	Report with optimized protocol, based on data from titrating four variables ([ionic], [$MgCl_2$], [Mant-GTP] and temp.).	DEV026	4
GTPase assay (abs, endpoint, 650nm)	GTP hydrolysis assay, detecting phosphate	Same as above, except [Mant-GTP] is replaced by [G-protein] and if available [GAP protein].	DEV031	4
Motor ATPase (abs, kinetic, 360nm)	ATP hydrolysis assay, detecting phosphate	Report with optimized protocol, based on data from titrating five variables ([ionic], [$MgCl_2$], [Motor], [microtubules] and temp.).	DEV034	4