Cytoskeleton, Inc.
The Protein Experts

Custom Services Newsletter

Q1 2014

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

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As Cytoskeleton's Custom Services Department continues to grow and expand its offerings, we wanted to take the opportunity to highlight some past research projects that benefited from work performed by Cytoskeleton's Custom Services scientists. The three main foci of Cytoskeleton's Custom Services are: 1) Compound Screening, 2) Assay Development, and 3) Gene Cloning and Recombinant/Native Protein Purification. The citations discussed below demonstrate our effectiveness in all three types of custom services.

Compound Screening

In Towle et al.[1], the anti-cancer activity of two compounds derived from halichondrin B were characterized under *in vitro* and *in vivo* conditions. Cytoskeleton's Custom Services Department participated in this study by screening the compounds for their effectiveness at inhibiting tubulin polymerization *in vitro*, as determined by IC50 calculations and comparisons against well-characterized tubulin polymerization inhibitors. The *in vitro* studies contributed to the conclusion that these compounds act as anti-cancer agents through an inhibition of tubulin polymerization[1]. The mammalian brain tubulin used in the screens was prepared by Cytoskeleton and used in an absorbance-based polymerization assay modified by Cytoskeleton scientists (Fig. 1A).

Assay Development and Compound Screening

Similarly, the second citation[2] involves purification of brain tubulin with or without microtubule-associated proteins (MAPs) along with purification of tubulin from HeLa cancer cells. Cytoskeleton's Custom Services scientists used these tubulins to evaluate the antimitotic effects of disorazol E1 using modified tubulin polymerization assays developed by Cytoskeleton. Cytoskeleton's tubulins and polymerization assays (Fig. 1B) were used to calculate IC50 values for this compound, helping to confirm its strong anticancer properties *in vitro* and qualifying it as a candidate for further *in vivo* anticancer studies[2].

Protein Purification and Assay Development

Tresch et al.[3] utilized Cytoskeleton's Custom Services to produce soybean tubulin and develop a modified tubulin polymerization assay. The authors used these reagents to investigate in detail the mode of action of the herbicides flamprop-M-methyl and its biologically active metabolite flamprop, focusing on the herbides' effects on *in vitro* plant (soybean) tubulin polymerization. Cytoskeleton, Inc. provided the >90% purified soybean tubulin and polymerization assay kit and protocols (Fig. 1C), significantly contributing to the novel finding that the herbicide flamprop-M-methyl has an antimicrotubule mechanism of action distinct from other herbicides. The classic herbicides work by disrupting microtubule assembly and stability, as demonstrated by *in vitro* tubulin polymerization assays. In contrast, flamprop-M-methyl does not affect plant tubulin polymerization *in vitro*; instead, flamprop-M-methyl exerts its herbicidal effects by affecting the organization and orientation of the mitotic spindle and phragmoblast microtubules[3].

These citations clearly demonstrate Cytoskeleton's commitment and ability to partner with a wide-range of scientists to pursue and complete their research projects with timely and unique contributions in the fields of compound screening, protein purification, and assay development. If you are interested in discussing how Cytoskeleton's Custom Services Department can help your research, send an email to tservice@cytoskeleton.com and let us help you advance your science.

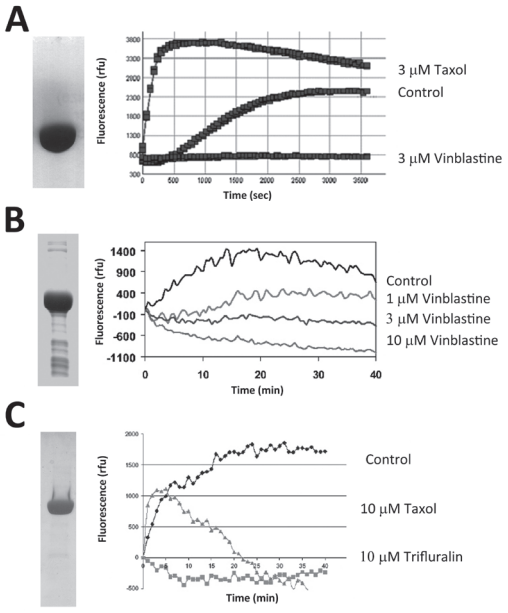


Figure 1. Purity of tubulin and polymerization curves. A. Purity of mammalian brain tubulin and polymerization activity with control buffer, taxol, or vinblastine. B. Purity of HeLa cancer cell tubulin and polymerization activity with control buffer and vinblastine. C. Purity of soybean tubulin and polymerization activity with control buffer, taxol, and trifluralin.

References

1. M.J. Towle et al. 2001. In vitro and in vivo anticancer activities of synthetic macrocyclic ketone analogues of halichondrin *B. Cancer Res*. **61**, 1013-1021.
2. S. Baasner et al. 2003. Disorazol E1 - A natural compound with outstanding in vitro antitumor activity. *American Association for Cancer Meeting*. Poster # 2117.
3. S. Tresch et al. 2008. The herbicide flamprop-M-methyl has a new antimicrotubule mechanism of action. *Pest Manag. Sci*. **64**, 1195-1203.

Custom Modules

Our recently expanded Custom Services 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 Services 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.

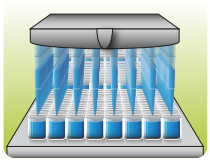
Clients Include:

- 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 ...

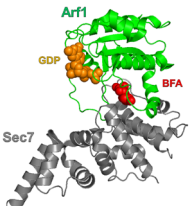
1. Choose a module and ask for a quote (24h turn around time)
2. Review quote, specifications, and deliverables
3. Place order and receive regular updates until project is finished

Compound Screening Modules



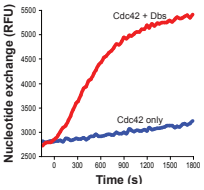
Type	Format	Deliverable	Module #	Timeline (wks)
Eg5 Kinesin motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	96 assays, consisting of 40 duplicate single concentrations (or 5 x IC50s), plus eight control wells. PDF Report with Executive Summary, Introduction, Methods, Results 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