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Helping advance science,
one protein at a time.

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Contents

Executive Summary

General Descriptions of the Field of Molecular Motors

Description of the Assays Used in Kinesin and Dynein Targeted Drug Discovery

Eukaryotic kinesin and dynein motor proteins orchestrate a wide range of kinetic events within a cell. They have been shown to move cargoes, such as chromosomes and vesicles, along microtubule tracks (1). They also play a major role in the organization of cytoskeletal architecture as evidenced in the establishment of the microtubule spindle during mitosis (2).
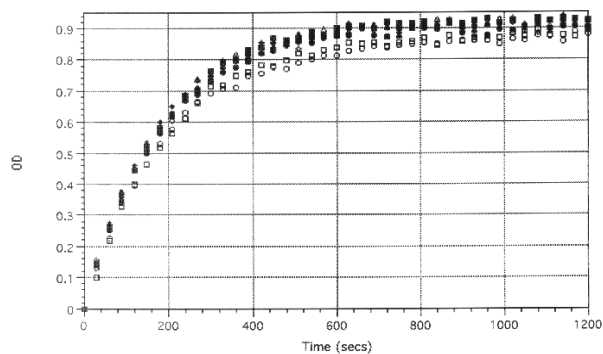
Kinesins operate by utilizing the energy of ATP hydrolysis to move along their microtubule (MT) substrates. Once a kinesin motor binds to its MT track, the ATPase rate of the motor is often enhanced several hundred to several thousand fold (3). MT activated kinesin ATPase is a major parameter in motor function and serves as a powerful method to monitor / study kinesin activity under various experimental conditions.

As part of it's **C**ytoskeleton **M**otor **W**erks (CMW) line of research reagents, Cytoskeleton Inc. has developed two assays to measure Microtubule Stimulated Kinesin ATPase. First the kinesin end-point assay is an extremely quick and economical way to measure inorganic phosphate (Pi) generated during the microtubule activated ATPase activity of kinesin motor proteins (Cat. # BK053). Large numbers of assays can be performed simultaneously in a homogenous reaction, making the assay highly suitable for HTS applications. The assay is an adaptation of the method of Kodama, T. et al. J. Biochem. 99: 1465-1472 (1986). Essentially liberated phosphate is detected by a malachite green / molybdate complex which turns a green color on binding to phosphate.

The kinetic kinesin ATPase assay is based on the Kinesin ELIPA (Enzyme Linked Inorganic Phosphate Assay, cat. # BK060) Biochem Kit offered by Cytoskeleton Inc. The assay is an adaptation of a method originally described by Webb for the measurement of glycerol kinase plus D-glyceraldehyde ATPase activity and for actin activated myosin ATPase (4). The assay is based upon an absorbance shift (330 - 360 nm) that occurs when 2-amino-6-mercapto-7-methylpurine ribonucleoside (MESG) is catalytically converted to 2-amino-6-mercapto-7-methyl purine in the presence of inorganic phosphate (Pi). The reaction is catalysed by purine nucleoside phosphorylase (PNP). One molecule of inorganic phosphate will yield one molecule of 2-amino-6-mercapto-7-methyl purine in an essentially irreversible reaction (5). Thus, the absorbance at 360 nm is directly proportional to the amount of Pi generated in the kinesin ATPase reaction. Figure 1 shows a typical set of results from a kinesin heavy chain motor domain protein MT activated ATPase ELIPA.

Figure 1   <u>Kinesin Heavy Chain ELIPA</u>

Method: The reactions were conducted in a 96 well plate format (300 ul reaction volumes). Each reaction contains 94 nM kinesin heavy chain motor domain protein (cat # KR01), 0.66 uM taxol stabilized microtubules (cat # MT001), 0.2 mM MESG, 0.3 U PNP, 15 uM taxol, 15 mM PIPES pH 7, 5 mM MgCl2, 0.6 mM ATP. Control reactions were carried out in the absence of motor protein and in the absence of MTs, these reactions gave readings of <0.02 (data not shown). Reactions were measured in a SpectraMax 250 (Molecular Devices) set in kinetic mode and 360 nm absorbance wavelength. Readings were taken at room temperature once every 30 seconds for a total reaction time of 20 minutes.

Method: Motor panel screening conditions.

Condition 1, compound preparation.

Prepare compounds by dilution into DMSO at 100x concentration. Pipette 1 µl of DMSO solution directly into each well.

Condition 2, reaction temperature.

Reactions were run at 22°C for all plates, except plate 3, which was run at 37°C.

Condition 3, reaction's "motor mix".

Mix the following in order at RT to obtain the "motor mix".

2 ml   15 mM Pipes-NaOH pH 7.0, 10 mM MgCl2, 20 µM Taxol (Buffer 1).

1 ml   5x MESG (ELIPA 1 reagent, Cat. # BK051).

0.2ml (Plates 1, 5, and 7) or 1 ml (Plates 2, 3, 4, and 6) 2.5 mg/ml MTs (1 x 10mg Cat. # MT002-XL resuspended in 4 ml of Buffer 1).

0.05 ml 100 x PNP (ELIPA 2 reagent, cat. # BK051).

Plate	Motor	Cat. #	Amount per 100 µl reaction (µg)
1	Kinesin heavy chain	KR01	0.20
1	CenPE	CP01	0.5
1	Chromokinesin	CR01	4.0
1	Eg5	EG01	2.0
2	MKLP2	CS-MP05	0.5
3	KIF7	CS-KF517	4.0
4	KIFC3	KC01	4.0
4	KIF3C	KF01	4.0
4	MKLP	MP01	1.0
5	KIF22	CS-KF02	2.0
6	KIF3C Repeat	KF01	4.0
6	MCAK	MK01	2.0
7	Kinesin heavy chain Repeat	KR01	0.20

Condition 4, reaction initiation.

Pipette 1.5ml of 3 mM ATP into each motor mix or 3 mM GTP for KIF7.

Pipette 100 µl into each well.

Condition 5, kinetic protocol.

Start protocol, 5-second rapid circular mixing, 41 readings, 30 seconds apart, 22°C, OD 360nm. For Plate 3, the temperature was increased to 37°C.

Notes:
High DMSO in the dilution of compounds is important to retain solubility.

Plate number	Cat #	Amount per reaction (ug)	Total ug required	Tubes of product required	Lot #
1 (high activity)	KR01	0.2	14.3	1 x 25 ug	038
1 (high activity)	CP01	0.5	12	1 x 25 ug	018
1 (high activity)	CR01	4	96	4 x 25 ug	011
1 (high activity)	EG01	2	48	2 x 25 ug	035
2 (low activity)	CS-MP05	0.5	12	1 x 50 ug	024
3 (low activity)	CS-KIF7 (needs GTP and 37C instead of ATP)	4	96	4 x 25 ug	013
4 (low activity)	KC01	4	96	4 x 25 ug	022
4 (low activity)	KF01	4	96	4 x 25 ug	013
4 (low activity)	MP01	1	24	1 x 25 ug	018
5 (high activity)	CS-KIF02	2	48	1 x 100 ug	013
6 (low activity)	KF01 Repeat	4	96	4 x 25 ug	013
6 (low activity)	MK01	2	48	2 x 25 ug	017
7 (high activity)	KR01 Repeat	0.2	14.3	1 x 25 ug	038

Table 1: Summary of results

Compound ID	Significant inhibition (>20% reduced activity compared to control)	Significant Enhancement (>20% increased activity compared to control)	<20% inhibition or enhancment detected	Notes
A1	Eg5	MKLP2	KHC, CENP-E, Chromokinesin, KIFC3, KIF3C, MCAK, MKLP1, KIF7, KIF22	
A2			KHC, CENP-E, Chromokinesin, Eg5, KIFC3, KIF3C, MCAK, MKLP1, MKLP2, KIF7, KIF22	
A3		Chromokinesin	KHC, CENP-E, Eg5, KIFC3, KIF3C, MCAK, MKLP1, MKLP2, KIF7, KIF22	
Monastrol (100 μM)	Eg5			Used only for Eg5 control
AMPPNP (1mM)	KHC, CENP-E, Chromokinesin, KIFC3, KIF3C, MCAK, MKLP1, MKLP2, KIF7, KIF22			

Motor: Kinesin HC (Cat# KR01)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	102.6	106.3		104.4	<mark>129.4</mark>
	3 µM	114.6	105.9		110.2	<mark>136.6</mark>
	0.3 µM	100.0	113.1		106.5	<mark>132.0</mark>
A2	10 µM	96.3	103.9		100.1	<mark>124.1</mark>
	3 µM	103.4	97.9		100.7	<mark>124.8</mark>
	0.3 µM	110.0	102.0		106.0	<mark>131.4</mark>
A3	10 µM	110.8	106.6		108.7	<mark>134.7</mark>
	3 µM	102.9	99.8		101.4	<mark>125.6</mark>
	0.3 µM	120.4	115.0		117.7	<mark>145.8</mark>
AMP-PNP	1 mM	8.7	12.2		10.5	13.0
DMSO	1%	81.1	82.5	78.5	80.7	100.0
No Motor		-1.7			-1.7	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: Kinesin HC (Cat# KR01) Repeat

Compound	Concentration (μM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 μM	121.5	110.6		116.1	116.9
	3 μM	123.4	111.6		117.5	118.4
	0.3 μM	103.0	110.3		106.6	107.4
A2	10 μM	107.9	100.1		104.0	104.8
	3 μM	106.7	106.3		106.5	107.3
	0.3 μM	105.1	107.6		106.3	107.1
A3	10 μM	110.9	105.3		108.1	108.9
	3 μM	111.5	110.2		110.9	111.7
	0.3 μM	109.2	115.8		112.5	113.3
AMP-PNP	1 mM	19.2	17.0		18.1	18.3
DMSO	1%	98.6	91.6	107.5	99.3	100.0
No Motor		6.0			6.0	6.0

<mark style="background-color:yellow">Significant enhancement</mark> (>20% increased activity compared to 1% DMSO control)
<mark style="background-color:red">Significant inhibition</mark> (>20% reduced activity compared to 1% DMSO control)

Motor: CENP-E (Cat# CP01)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	109.9	102.6		106.2	111.3
	3 µM	95.4	94.4		94.9	99.4
	0.3 µM	103.9	97.2		100.6	105.3
A2	10 µM	87.9	97.9		92.9	97.3
	3 µM	108.8	96.5		102.6	107.5
	0.3 µM	114.8	106.5		110.7	115.9
A3	10 µM	89.6	84.7		87.2	91.3
	3 µM	95.4	119.9		107.7	112.8
	0.3 µM	113.4	107.6		110.5	115.7
AMP-PNP	1 mM	15.0	13.9		14.4	15.1
DMSO	1%	97.4	94.4	94.7	95.5	100.0
No Motor		-8.2			-8.2	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: Chromokinesin (Cat# CR01)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	34.0	28.1		31.1	97.0
	3 µM	26.0	28.6		27.3	85.2
	0.3 µM	29.5	28.1		28.8	90.0
A2	10 µM	33.8	32.6		33.2	103.7
	3 µM	26.2	26.8		26.5	82.8
	0.3 µM	35.1	25.2		30.2	94.2
A3	10 µM	55.4	56.4		55.9	174.3
	3 µM	40.5	38.1		39.3	122.7
	0.3 µM	37.3	29.3		33.3	104.0
AMP-PNP	1 mM	15.8	20.7		18.3	57.0
DMSO	1%	27.2	33.7	35.2	32.0	100.0
No Motor		-6.2			-6.2	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: Eg5 (Cat# EG01)

Compound	Concentration (μM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 μM	56.5	61.3		58.9	72.9
	3 μM	70.1	70.8		70.5	87.2
	0.3 μM	96.5	87.7		92.1	114.0
A2	10 μM	82.6	84.5		83.6	103.4
	3 μM	86.5	73.3		79.9	98.9
	0.3 μM	85.8	86.4		86.1	106.6
A3	10 μM	64.9	69.5		67.2	83.2
	3 μM	76.8	83.0		79.9	98.9
	0.3 μM	84.4	80.1		82.3	101.8
Monastrol	100 μM	4.7	5.8		5.3	6.5
DMSO	1%	81.7	83.9	76.7	80.8	100.0
No Motor		-4.7			-4.7	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: KIF22 (Cat# KF02)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	180.6	161.9		171.2	102.7
	3 µM	152.7	157.1		154.9	92.9
	0.3 µM	152.0	157.8		154.9	92.9
A2	10 µM	154.8	166.8		160.8	96.4
	3 µM	164.7	173.2		168.9	101.3
	0.3 µM	167.1	166.0		166.6	99.9
A3	10 µM	170.0	185.6		177.8	106.7
	3 µM	162.7	163.5		163.1	97.8
	0.3 µM	152.8	169.1		161.0	96.6
AMP-PNP	1 mM	73.0	77.8		75.4	<mark>45.2</mark>
DMSO	1%	160.1	165.8	174.2	166.7	100.0
No Motor		-2.7			-2.7	<mark>0</mark>

<mark>Significant enhancement</mark> (>20% increased activity compared to 1% DMSO control)
<mark>Significant inhibition</mark> (>20% reduced activity compared to 1% DMSO control)

Motor: KIF3C (Cat# KF01)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	31.2	28.1		29.6	103.8
	3 µM	29.6	28.8		29.2	102.3
	0.3 µM	27.8	27.3		27.6	96.5
A2	10 µM	37.5	29.8		33.6	117.8
	3 µM	30.0	30.3		30.1	105.5
	0.3 µM	28.2	29.4		28.8	100.9
A3	10 µM	27.6	29.9		28.8	100.8
	3 µM	29.7	28.3		29.0	101.6
	0.3 µM	25.9	27.9		26.9	94.3
AMP-PNP	1 mM	33.4	22.5		28.0	98.0
DMSO	1%	28.6	28.8	28.3	28.5	100.0
No Motor		-5.4			-5.4	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: KIF3C (Cat# KF01)
Repeat

Compound	Concentration (μM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 μM	24.5	24.0		24.3	100.1
	3 μM	25.9	24.3		25.1	103.5
	0.3 μM	24.2	23.7		24.0	98.8
A2	10 μM	25.7	23.8		24.7	102.0
	3 μM	24.8	24.1		24.4	100.7
	0.3 μM	25.1	24.3		24.7	101.8
A3	10 μM	23.7	23.6		23.6	97.4
	3 μM	23.8	22.8		23.3	95.9
	0.3 μM	23.7	24.3		24.0	98.9
AMP-PNP	1 mM	12.6	16.0		14.3	58.9
DMSO	1%	24.0	23.9	24.9	24.3	100.0
No Motor		-1.9			-1.9	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: KIFC3 (Cat# KC01)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	26.4	26.3		26.4	106.6
	3 µM	26.4	29.8		28.1	113.6
	0.3 µM	22.7	23.4		23.0	93.0
A2	10 µM	24.0	28.2		26.1	105.4
	3 µM	26.7	29.9		28.3	114.5
	0.3 µM	23.8	26.6		25.2	101.9
A3	10 µM	25.2	24.7		24.9	100.8
	3 µM	28.3	25.5		26.9	108.7
	0.3 µM	24.8	30.1		27.5	111.0
AMP-PNP	1 mM	4.4	8.7		6.6	26.5
DMSO	1%	26.4	25.0	22.8	24.7	100.0
No Motor		-1.2			-1.2	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: MCAK (Cat# MK01)						
Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	22.8	16.8		19.8	93.8
	3 µM	20.4	22.5		21.4	101.3
	0.3 µM	20.7	24.5		22.6	106.8
A2	10 µM	19.5	25.5		22.5	106.4
	3 µM	19.7	25.4		22.5	106.6
	0.3 µM	27.7	28.3		28.0	132.6
A3	10 µM	24.2	26.0		25.1	118.8
	3 µM	25.7	22.3		24.0	113.3
	0.3 µM	23.9	21.0		22.5	106.4
AMP-PNP	1 mM	12.2	9.1		10.7	50.4
DMSO	1%	21.3	18.7	23.4	21.1	100.0
No Motor		-3.4			-3.4	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Motor: MKLP1 (Cat# MP01)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	**10 µM**	**130.8**	**114.5**		**122.6**	**95.7**
	3 µM	**134.9**	**127.8**		**131.4**	**102.6**
	0.3 µM	**123.0**	**140.4**		**131.7**	**102.8**
A2	**10 µM**	**132.9**	**131.7**		**132.3**	**103.2**
	3 µM	**126.8**	**131.5**		**129.1**	**100.8**
	0.3 µM	**132.3**	**126.2**		**129.3**	**100.9**
A3	**10 µM**	**119.4**	**116.8**		**118.1**	**92.2**
	3 µM	**132.9**	**128.9**		**130.9**	**102.2**
	0.3 µM	**115.6**	**131.8**		**123.7**	**96.6**
AMP-PNP	**1 mM**	**44.1**	**54.7**		**49.4**	<mark style="background-color:red">**38.6**</mark>
DMSO	**1%**	**136.4**	**133.2**	**114.6**	**128.1**	**100.0**
No Motor		**3.2**			**3.2**	<mark style="background-color:red">**2.5**</mark>

<mark style="background-color:yellow">Significant enhancement</mark> (>20% increased activity compared to 1% DMSO control)
<mark style="background-color:red">Significant inhibition</mark> (>20% reduced activity compared to 1% DMSO control)

Motor: MKLP2 (Cat# MP05)

Compound	Concentration (µM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 µM	29.3	31.5		30.4	<mark>125.7</mark>
	3 µM	30.3	29.0		29.7	<mark>122.6</mark>
	0.3 µM	35.5	25.3		30.4	<mark>125.7</mark>
A2	10 µM	30.9	21.6		26.3	108.5
	3 µM	27.3	26.5		26.9	111.0
	0.3 µM	32.1	24.4		28.2	116.6
A3	10 µM	22.5	22.9		22.7	93.7
	3 µM	25.0	26.6		25.8	106.6
	0.3 µM	28.3	28.9		28.6	118.1
AMP-PNP	1 mM		-5.0		-5.0	0
DMSO	1%	24.4	23.7	24.4	24.2	100.0
No Motor		-9.1			-9.1	0

<mark>Significant enhancement</mark> (>20% increased activity compared to 1% DMSO control)
<mark>Significant inhibition</mark> (>20% reduced activity compared to 1% DMSO control)

Motor: KIF7 (Cat# CS-KF51)

Compound	Concentration (μM)	Run 1	Run 2	Run 3	Average (milli units per min)	% Activity
A1	10 μM	36.2	34.9		35.6	98.0
	3 μM	35.0	34.3		34.6	95.5
	0.3 μM	34.0	33.6		33.8	93.2
A2	10 μM	33.6	34.1		33.8	93.2
	3 μM	35.0	35.8		35.4	97.6
	0.3 μM	34.3	34.8		34.6	95.3
A3	10 μM	34.7	33.3		34.0	93.8
	3 μM	36.3	34.3		35.3	97.3
	0.3 μM	33.8	35.3		34.6	95.3
AMP-PNP	1 mM	16.7	35.8		26.2	72.3
DMSO	1%	36.2	35.3	37.4	36.3	100.0
No Motor		-5.2			-5.2	0

Significant enhancement (>20% increased activity compared to 1% DMSO control)
Significant inhibition (>20% reduced activity compared to 1% DMSO control)

Plate 1 Layout

| | Compound A1 | | | Compound A2 | | | Compound A3 | | | | | |
| | 10 µM | 3 µM | 0.3 µM | 10 µM | 3 µM | 0.3 µM | 10 µM | 3 µM | 0.3 µM | | | |
	1	2	3	4	5	6	7	8	9	10	11	12
KHC A	01	**1** 01	01	01	**2** 01	01	01	**3** 01	01	**AMPPNP** 01	01	**KR01** 02
B	01	01	01	01	01	01	01	01	01	01	01	**NoMotor** 01
CENP-E C	01	01	01	01	01	01	01	01	01	01	01	**CP01** 01
D	01	01	01	01	01	01	01	01	01	01	01	**NoMotor** 02
Chromokinesin E	01	01	01	01	01	01	01	01	01	01	01	**CR01** 01
F	01	01	01	01	01	01	01	01	01	01	01	**NoMotor** 03
Eg5 G	01	01	01	01	01	01	01	01	01	**Mona** 01	01	**Eg5** 01
H	01	01	01	01	01	01	01	01	01	01	01	**NoMotor** 03

Plate1

	1	2	3	4	5	6	7	8	9	10	11	12
A	102.62	114.58	99.966	96.349	103.44	110.03	110.77	102.95	120.36	8.714	81.079	78.471
B	106.27	105.89	113.08	103.86	97.907	101.99	106.61	99.789	115.00	12.228	82.517	-1.737
C	109.90	95.449	103.91	87.898	108.78	114.77	89.628	95.414	113.41	14.965	97.382	94.703
D	102.60	94.353	97.219	97.875	96.498	106.55	84.748	119.92	107.59	13.897	94.365	-8.222
E	34.050	25.965	29.498	33.838	26.224	35.116	55.350	40.500	37.324	15.833	27.197	35.171
F	28.094	28.613	28.145	32.621	26.821	25.226	56.358	38.150	29.316	20.719	33.748	-6.217
G	56.518	70.103	96.462	82.610	86.475	85.844	64.933	76.835	84.413	4.749	81.733	76.724
H	61.296	70.827	87.688	84.531	73.331	86.376	69.474	83.018	80.108	5.827	83.925	-4.745

Settings Information

◢ Kinetic
Time: 20:00
Interval: 00:00:30
Reads: 41
◢ Absorbance
Lm1 360
◢ Plate Type
96 Well Corning Half Area flat cl
Height 14.2 mm
◢ More Settings
Shake before 5s Orb., Med.
Shake between Off
Speed Read On
ReadOrder Row

Read Information
SpectraMax iD5

22

Plate 2 Layout

		Compound A1			Compound A2			Compound A3					
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM			
		1	2	3	4	5	6	7	8	9	10	11	12
MKLP2	A	01	01	01	01	01	01 MP05	01	01	01	AMP-PNP	DMSO	DMSO
	B	01	01	01	01	01	01	01	01	01	AMP-PNP	DMSO	No Motor 01
	C												
	D												
	E												
	F												
	G												
	H												

Plate 2 MP05

	1	2	3	4	5	6	7	8	9	10	11	12
A	29.324	30.296	35.480	30.933	27.259	32.051	22.454	25.032	28.260	83.932	24.391	24.445
B	31.492	29.033	25.320	21.574	26.454	24.361	22.873	26.555	28.898	-5.049	23.743	-9.050
C	0.238	0.147	0.314	0.205	0.262	-0.229	-0.219	0.252	0.314	-0.281	0.247	0.239
D	0.423	0.364	0.189	-0.255	0.296	0.215	0.175	0.228	0.308	0.350	0.426	-0.250
E	0.200	0.315	0.219	0.431	-0.350	0.378	0.201	0.331	0.201	-0.269	0.332	0.258
F	0.231	0.417	0.244	0.207	0.229	0.260	-0.257	0.253	-0.288	0.210	-0.352	-0.260
G	0.279	-0.210	0.188	0.227	-0.319	0.318	-0.172	0.380	0.281	-0.181	-0.231	0.174
H	0.295	-0.400	0.328	0.346	0.200	-0.243	0.314	0.346	0.207	-0.212	-0.200	0.257

Plate 3 Layout

	Compound A1			Compound A2			Compound A3					
	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM			
	1	2	3	4	5	6	7	8	9	10	11	12
A	03	03	03	03	03	03	03	03	03	AMP-PNP	DMSO	DMSO
B	03	03	03	03	03	03	03	03	03	AMP-PNP	DMSO	No Motor
C												
D												
E												
F												
G												
H												

KIF7

Kif7

Plate 3 Kif7 R

	1	2	3	4	5	6	7	8	9	10	11	12
A	36.166	35.014	34.033	33.594	35.043	34.350	34.679	36.293	33.833	16.709	36.235	37.351
B	34.943	34.276	33.558	34.054	35.804	34.823	33.348	34.274	35.296	35.760	35.255	-5.201
C												
D												
E												
F												
G												
H												

Settings Information

◢ Kinetic
Time: 20:00
Interval: 00:00:30
Reads: 41
◢ Absorbance
Lm1   360
◢ Plate Type
96 Well Corning Half Area flat cl
Height 14.2 mm
◢ More Settings
Shake before 5s Orb., Med.
Shake between Off
Speed Read On
ReadOrder Row

Read Information

SpectraMax iD5

Plate 4 Layout

		Compound A1			Compound A2			Compound A3					
		10 μM	3 μM	0.3 μM	10 μM	3 μM	0.3 μM	10 μM	3 μM	0.3 μM			
		1	2	3	4	5	6	7	8	9	10	11	12
KIFC3	A	02	02	02	02	02	02 (KC01)	02	02	02	AMP-PNP	DMSO	DMSO
	B	02	02	02	02	02	02	02	02	02	AMP-PNP	DMSO	No Motor
KIF3C	C	02	02	02	02	02	02 (KF01)	02	02	02	AMP-PNP	DMSO	DMSO
	D	02	02	02	02	02	02	02	02	02	AMP-PNP	DMSO	No Motor
	E										AMP-PNP	DMSO	DMSO
	F										AMP-PNP	DMSO	No Motor
MKLP1	G	02	02	02	02	02	02 (MP01)	02	02	02	AMP-PNP	DMSO	DMSO
	H	02	02	02	02	02	02	02	02	02	AMP-PNP	DMSO	No Motor

Plate 4 KC01 KF01 MK01 MP01R

	1	2	3	4	5	6	7	8	9	10	11	12
A	26.429	26.437	22.662	23.979	26.719	23.789	25.236	28.299	24.826	4.387	26.416	22.791
B	26.339	29.753	23.360	28.194	29.942	26.630	24.653	25.465	30.085	8.743	25.016	-1.238
C	31.219	29.608	27.812	37.463	29.962	28.192	27.636	29.665	25.876	33.448	28.563	28.270
D	28.062	28.776	27.297	29.795	30.261	29.379	29.933	28.329	27.947	22.502	28.795	-5.375
E	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked
F	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked	Masked
G	130.78	134.93	122.98	132.86	126.76	132.35	119.44	132.88	115.65	44.073	136.42	114.61
H	114.49	127.79	140.42	131.65	131.49	126.24	116.80	128.94	131.78	54.687	133.25	3.173

Settings Information

▲ Kinetic
Time: 20:00
Interval: 00:00:30
Reads: 41
▲ Absorbance
Lm1   360
▲ Plate Type
96 Well Corning Half Area flat
Height 14.2 mm
▲ More Settings
Shake before 5s Orb., Med.
Shake between Off
Speed Read On
ReadOrder Row

Read Information
SpectraMax iD5

Plate 5 Layout

	Compound A1			Compound A2			Compound A3					
	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM			
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G	01	01	01	01	01	01	01	01	01	AMP-PNP	DMSO	DMSO
H	01	01	01	01	01	01	01	01	01	AMP-PNP	DMSO	No Motor

KIF22

KIF22

Plate 5 KIF22

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.324	-1.878	-1.147	-5.331	-4.507	-4.635	-5.123	-5.799	-3.614	0.991	-1.069	-0.490
B	-0.767	-3.284	-2.101	-2.343	-1.874	-2.072	1.845	-1.806	-1.888	-2.142	-2.906	5.132
C	0.314	-2.137	-1.255	2.009	2.154	1.437	-2.021	0.910	1.662	4.509	-1.037	-0.499
D	-0.539	-1.728	-0.992	-1.307	1.524	1.593	-1.243	-0.902	-1.007	4.819	-1.456	-0.774
E	-4.363	-3.530	-3.447	-3.783	-3.317	-3.637	-3.848	-3.398	-3.152	-2.557	-3.262	-4.149
F	-3.231	-2.853	-3.782	-2.604	-3.216	-2.776	-3.040	-2.669	-3.763	-2.464	-3.102	-3.778
G	180.61	152.75	152.00	154.76	164.66	167.12	170.00	162.69	152.84	72.987	160.06	174.21
H	161.89	157.14	157.79	166.79	173.15	166.03	185.60	163.50	169.14	77.843	165.82	-2.655

Settings Information

⊿ Kinetic

Time: 20:00

Interval: 00:00:30

Reads: 41

⊿ Absorbance

Lm1   360

⊿ Plate Type

96 Well Corning Half Area flat cl

Height 14.2 mm

⊿ More Settings

Shake before 5s Orb., Med.

Shake between Off

Speed Read On

ReadOrder Row

Read Information

SpectraMax iD5

Plate 6 Layout

Plate 6 KF01 MK01

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	24.513	25.920	24.203	25.676	24.752	25.090	23.677	23.769	23.658	12.606	24.000	24.906
F	24.033	24.289	23.721	23.803	24.086	24.319	23.565	22.772	24.309	15.983	23.874	-1.905
G	22.818	20.380	20.702	19.489	19.656	27.723	24.235	25.652	23.948	12.160	21.276	23.419
H	16.837	22.455	24.464	25.515	25.428	28.342	25.995	22.267	21.034	9.148	18.749	-3.447

Settings Information

◢ Kinetic
Time: 20:00
Interval: 00:00:30
Reads: 41
◢ Absorbance
Lm1   360
◢ Plate Type
96 Well Corning Half Area flat
Height 14.2 mm
◢ More Settings
Shake before 5s Orb., Med.
Shake between Off
Speed Read On
ReadOrder Row

Read Information
SpectraMax iD5

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Plate 7 Layout

	Compound A1			Compound A2			Compound A3					
	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM			
	1	2	3	4	5	6	7	8	9	10	11	12
A	01	01	01	01	01	01 (KHC)	01	01	01	AMP-PNP	DMSO	DMSO
B	01	01	01	01	01	01	01	01	01	AMP-PNP	DMSO	No Motor
C												
D												
E												
F												
G												
H												

KHC

Plate7 KHC repeat

	1	2	3	4	5	6	7	8	9	10	11	12
A	121.51	123.43	102.99	107.86	106.74	105.06	110.89	111.48	109.19	19.234	98.622	107.54
B	110.61	111.59	110.29	100.13	106.32	107.56	105.29	110.23	115.78	17.016	91.601	5.976
C	0.093	0.162	0.179	-0.264	0.150	0.150	0.198	0.167	0.152	0.236	-0.133	0.110
D	0.109	0.112	-0.119	-0.167	0.162	0.200	0.148	0.157	-0.141	0.131	0.155	0.214
E	0.186	-0.136	0.133	-0.209	0.155	0.148	0.143	0.105	0.172	0.131	0.136	-0.138
F	-0.188	0.112	-0.150	-0.167	-0.126	0.136	-0.188	0.157	-0.225	0.138	-0.117	0.095
G	-0.098	0.126	-0.086	0.162	-0.153	-0.127	0.121	-0.171	0.107	-0.117	-0.200	0.136
H	0.153	-0.198	0.241	-0.164	-0.208	-0.169	-0.176	0.143	-0.102	0.119	0.210	0.152

Settings Information

◢ Kinetic

Time: 20:00

Interval: 00:00:30

Reads: 41

◢ Absorbance

Lm1   360

◢ Plate Type

96 Well Corning Half Area flat

Height 14.2 mm

◢ More Settings

Shake before 5s Orb., Med.

Shake between Off

Speed Read On

ReadOrder Row

Read Information

SpectraMax iD5

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Plate 1

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO			
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM					
Raw Vmax (slopes)		1	2	3	4	5	6	7	8	9	10	11	12		
A		102.6201	114.5792	99.96631	96.34939	103.4445	110.0251	110.771	102.9456	120.357	8.714122	81.07921	78.47075		**KR01 DMSO Avg**
B		106.2683	105.8946	113.0837	103.8574	97.9073	101.9914	106.6071	99.78898	114.9989	12.22782	82.51663	-1.73651		80.68886514
C		109.8995	95.4487	103.908	87.89767	108.7828	114.7653	89.62781	95.41409	113.4105	14.9655	97.38212	94.70315		**CP01 DMSO Avg**
D		102.598	94.35314	97.21877	97.87515	96.49796	106.5486	84.7479	119.9201	107.5935	13.89741	94.36502	-8.22245		95.48342982
E		34.04974	25.96451	29.49751	33.83804	26.22355	35.11613	55.35013	40.50032	37.32391	15.83269	27.19731	35.17109		**CR01 DMSO Avg**
F		28.09427	28.61303	28.14471	32.62097	26.82059	25.22574	56.35845	38.14972	29.31617	20.71852	33.74799	-6.21738		32.03879802
G		56.51772	70.10273	96.46221	82.61008	86.47524	85.84381	64.93296	76.83505	84.41251	4.748538	81.73257	76.72354		**Eg5 DMSO Avg**
H		61.29625	70.82719	87.68776	84.53133	73.33138	86.37624	69.47421	83.01784	80.10785	5.827479	83.92536	-4.74458		80.79382334
100*(Vmax/DMSO Avg)		1	2	3	4	5	6	7	8	9	10	11	12		
A		127.18	142.0012	123.8911	119.4085	128.2017	136.3572	137.2816	127.5834	149.1619	10.79966	100.4838	97.25103	**KR01**	
B		131.7014	131.2382	140.1478	128.7134	121.3393	126.4008	132.1212	123.6713	142.5214	15.15428	102.2652	-2.15211		
C		115.098	99.96362	108.8231	92.05542	113.9285	120.194	93.86739	99.92738	118.7751	15.6734	101.9885	99.18281	**CP01**	
D		107.4511	98.81625	101.8174	102.5049	101.0625	111.5885	88.75666	125.5926	112.6829	14.55479	98.82869	-8.61139		
E		106.2766	81.04083	92.06811	105.6158	81.84934	109.605	172.7597	126.4102	116.496	49.41725	84.88867	109.7766	**CR01**	
F		87.68826	89.30743	87.84571	101.8171	83.71286	78.73497	175.9069	119.0735	91.50209	64.66697	105.3348	-19.4058		
G		69.95302	86.76744	119.3931	102.248	107.032	106.2505	80.36872	95.10016	104.4789	5.877353	101.1619	94.96213	**Eg5**	
H		75.8675	87.66412	108.5328	104.626	90.7636	106.9095	85.9895	102.7527	99.15095	7.212777	103.876	-5.87246		

Plate 2

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO			
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM					
Raw Vmax (slopes)		1	2	3	4	5	6	7	8	9	10	11	12		
A		29.32358	30.29561	35.4797	30.93325	27.25915	32.05053	22.45377	25.03179	28.25983	83.93151	24.39051	24.44469		**MP05 DMSO Avg**
B		31.49195	29.03284	25.31976	21.57386	26.45373	24.36058	22.87298	26.55506	28.89752	-5.04887	23.74338	-9.05015		24.19285766
C															
D															
E															
F															
G															
H															
100*(Vmax/DMSO Avg)															
A		121.2076	125.2254	146.6536	127.8611	112.6744	132.4793	92.81155	103.4677	116.8106	346.9268	100.817	101.0409		
B		130.1705	120.0058	104.658	89.1745	109.3452	100.6933	94.54434	109.7641	119.4465	-20.8693	98.14209	-37.4083		
C															
D															
E															
F															
G															
H															

Plate 3

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO			
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM					
Raw Vmax (slopes)		1	2	3	4	5	6	7	8	9	10	11	12		
A															
B															
C		36.16635	35.01431	34.03334	33.59415	35.04296	34.34995	34.67886	36.29274	33.83326	16.70906	36.23473	37.35096		KF-51 Avg
D		34.94277	34.27565	33.55767	34.05366	35.80423	34.82307	33.3481	34.27371	35.29627	35.75957	35.25516	-5.201		36.28028031
E															
F															
G															
H															
100*(Vmax/DMSO Avg)															
A															
B															
C		99.68597	96.51057	93.80671	92.59616	96.58955	94.67938	95.58598	100.0343	93.25522	46.05547	99.87444	102.9511		
D		96.3134	94.4746	92.49561	93.86272	98.68785	95.98347	91.91797	94.46924	97.28775	98.56476	97.17443	-14.3356		
E															
F															
G															
H															

Plate 4

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO			
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM					
Raw Vmax (slopes)		1	2	3	4	5	6	7	8	9	10	11	12		
A		26.42862	26.43741	22.6621	23.97859	26.71913	23.78929	25.23567	28.29933	24.82609	4.387307	26.41639	22.79061		**KC01 DMSO Avg**
B		26.33894	29.75337	23.36	28.19388	29.94206	26.63002	24.65324	25.46531	30.08479	8.743117	25.01601	-1.23834		24.74100238
C		31.21887	29.60752	27.81213	37.46334	29.96164	28.19201	27.63597	29.66455	25.87567	33.44768	28.56259	28.27006		**KF01 DMSO Avg**
D		28.06232	28.77572	27.29653	29.79482	30.2614	29.37927	29.9334	28.32916	27.94733	22.50185	28.79487	-5.37533		28.54250501
E															
F															
G		130.7757	134.928	122.982	132.8554	126.7646	132.348	119.4383	132.8836	115.6465	44.07271	136.4152	114.6088		**MP01 DMSO Avg**
H		114.4916	127.7897	140.4187	131.6501	131.4881	126.2422	116.8017	128.9353	131.7781	54.68677	133.2482	3.172964		128.0907426
100*(Vmax/DMSO Avg)		1	2	3	4	5	6	7	8	9	10	11	12		

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO		
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM				
	A	106.8211	106.8567	91.59736	96.91841	107.9953	96.15329	101.9994	114.3823	100.3439	17.73294	106.7717	92.11676	KC01
	B	106.4587	120.2594	94.41818	113.9561	121.022	107.6352	99.64528	102.9275	121.5989	35.33857	101.1115	-5.00522	
	C	109.3768	103.7313	97.44111	131.2546	104.972	98.77202	96.8239	103.9312	90.65663	117.1855	100.0704	99.04549	KF01
	D	98.31766	100.8171	95.63467	104.3876	106.0222	102.9316	104.873	99.25255	97.91478	78.83627	100.8842	-18.8327	
	E													
	F													
	G	102.0962	105.3378	96.0116	103.7197	98.96472	103.3237	93.24508	103.7417	90.2848	34.40741	106.4989	89.47473	MP01
	H	89.38323	99.76496	109.6244	102.7788	102.6523	98.55683	91.18665	100.6593	102.8787	42.69377	104.0264	2.477122	

Plate 5

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO		
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM				
Raw Vmax (slopes)		1	2	3	4	5	6	7	8	9	10	11	12	
	A													
	B													
	C													
	D													
	E													
	F													
	G	180.6062	152.7465	151.9953	154.7564	164.6567	167.1218	170.0034	162.6872	152.8353	72.987	160.0565	174.2105	KIF22 DMSO Avg
	H	161.8928	157.1397	157.7876	166.7894	173.1542	166.0343	185.5961	163.4955	169.1392	77.84255	165.822	-2.65543	166.6963395
100*(Vmax/DMSO Avg)		1	2	3	4	5	6	7	8	9	10	11	12	
	A													
	B													
	C													
	D													
	E													
	F													
	G	108.3444	91.63157	91.18096	92.83731	98.77641	100.2553	101.9839	97.59494	91.68486	43.78441	96.0168	104.5077	KIF22
	H	97.11839	94.26703	94.65569	100.0558	103.8741	99.60285	111.3378	98.07984	101.4655	46.69721	99.47549	-1.59297	

Plate 6

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO		
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM				
Raw Vmax (slopes)		1	2	3	4	5	6	7	8	9	10	11	12	
	A													
	B													
	C													
	D													
	E	24.51281	25.92022	24.20304	25.67625	24.75238	25.08952	23.67718	23.76913	23.65837	12.60581	24.00005	24.90598	KF01 DMSO Avg
	F	24.03298	24.28856	23.72066	23.80321	24.08576	24.31883	23.56521	22.77152	24.30899	15.98347	23.87416	-1.90462	24.26006544
	G	22.81795	20.37976	20.70179	19.48863	19.65564	27.72327	24.23508	25.65211	23.9482	12.1603	21.27628	23.41925	MK01 DMSO Avg
	H	16.8372	22.45537	24.46421	25.51515	25.42849	28.34238	25.99499	22.26671	21.034	9.148083	18.74881	-3.44675	21.1481145
100*(Vmax/DMSO Avg)		1	2	3	4	5	6	7	8	9	10	11	12	
	A													
	B													
	C													
	D													
	E	101.0418	106.8431	99.76492	105.8375	102.0293	103.419	97.59736	97.97637	97.51981	51.96115	98.92823	102.6625	KF01
	F	99.06396	100.1175	97.77656	98.11684	99.28151	100.2422	97.1358	93.86422	100.2017	65.88385	98.4093	-7.85085	
	G	107.8959	96.36681	97.88951	92.15303	92.94276	131.091	114.5969	121.2974	113.2404	57.50066	100.6061	110.7392	MK01
	H	79.61561	106.1814	115.6803	120.6497	120.24	134.0185	122.9187	105.2893	99.46038	43.2572	88.65477	-16.2981	

Plate 7

		Cmpd 1			Cmpd 2			Cmpd 3			Inhib	DMSO		
		10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM	10 µM	3 µM	0.3 µM				
Raw Vmax (slopes)		1	2	3	4	5	6	7	8	9	10	11	12	
	A	121.5148	123.4279	102.9913	107.8579	106.7395	105.0574	110.8931	111.4778	109.1938	19.23434	98.62177	107.5374	KHC DMSO Avg
	B	110.6069	111.5873	110.2949	100.126	106.3202	107.5606	105.2899	110.2252	115.7846	17.01553	91.60052	5.976454	99.25323224
	C													
	D													
	E													
	F													
	G													
	H													
100*(Vmax/DMSO Avg)		1	2	3	4	5	6	7	8	9	10	11	12	
	A	150.5967	152.9677	127.6401	133.6714	132.2852	130.2007	137.433	138.1576	135.327	23.83767	122.2248	133.2742	
	B	137.0783	138.2933	136.6916	124.089	131.7657	133.3029	130.4888	136.6052	143.4951	21.08783	113.5231	7.406789	
	C													
	D													
	E													
	F													
	G													
	H													