ClariCELL™ Kinase Cellular Assay Results
Report number – XXXXXXXX-XX
Date

This report details compound testing results in the ClariCELL™ kinase cellular autophosphorylation assay(s) for the following compounds: CMPD-1, CMPD-2, CMPD-3, and CMPD-4. CNTRL CMPD was also tested as a control for the assay(s).

Short Assay Protocol

- HEK293 human embryonic kidney cells were transiently transfected with a vector encoding full-length human wild type kinase(s).
- Cells were dispensed into 96-well plates at an appropriate cell number previously determined and optimized by titration.
- Eight two- or three-fold serial dilutions were prepared for each compound in 100% DMSO.
- Compounds were then diluted in cell culture medium to 10x final assay concentration and 5% DMSO.
- Compounds were added to the cells in 96-well plates (10-fold dilution in cell culture medium) for a final concentration of 1x compound and 0.5% DMSO. For positive (high signal) controls, cells were treated with 0.5% DMSO alone. Negative (low signal) controls were either 1) cells transiently expressing kinase-deficient mutant kinase and treated with 0.5% DMSO, or 2) cells expressing wt kinase and treated with 1uM control inhibitor including 0.5% DMSO final concentration.
- Cells were incubated with compound at 37°C for 2 hours.
- Cells were lysed, and lysates were transferred to an ELISA plate that had been previously coated with antibody to capture the substrate (human kinase).
- Plates were washed then incubated with an antibody appropriate to detect phosphorylation at the relevant site(s).
- Plates were washed then incubated with HRP-linked secondary antibody.
- Plates were washed, then HRP substrate was added. Absorbance was read at 450nm.
- % inhibition values were calculated from absorbance readings based on positive and negative control values, and according to the following formula: (%INH = ((positive control – sample)/(positive control – negative control))*100
- Z' values were calculated based on the following formula: 1-[(3*standard deviation of positives + 3*standard deviation of negatives)/(average positive – average negative)]
- % inhibition values were plotted vs. the log of compound concentrations utilizing GraphPad Prism software.
- IC50s were determined after utilizing sigmoidal dose response curve fitting to generate the curves.

Compound Testing Template and Cell Plating

Cell plating and compound locations:

Plate 1:

	Control	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	Control
	1	2	3	4	5	6	7	8	9	10	11	12
A	POS	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	NEG
B	POS	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	NEG
C	POS	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	NEG
D	POS	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	NEG
E	NEG	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	POS
F	NEG	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	POS
G	NEG	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	POS
H	NEG	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	POS

Compound concentrations:

	[CMPD-1, -3] uM	[CMPD-2] uM	[CMPD-4] uM	[cntrl cmpd] uM
A	1	10	25	1
B	0.33	3.3	12.5	0.33
C	0.11	1.11	6.25	0.11
D	0.037	0.37	3.13	0.037
E	0.012	0.12	1.56	0.012
F	0.0041	0.041	0.78	0.0041
G	0.0014	0.014	0.39	0.0014
H	0.00046	0.0046	0.20	0.00046

Raw data (Absorbance at 450nM)

plate 1:

	Control	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	Control
	1	2	3	4	5	6	7	8	9	10	11	12
A	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm
B	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm
C	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm
D	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm
E	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm
F	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm
G	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm
H	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm	ABS 450nm

plate statistics for plate 1:

avg pos	value
avg neg	value
st dev pos	value
st dev neg	value
s/b	value
z'	value

Notes on Precipitation

No compounds had visible precipitation upon aqueous dilution.

% Inhibition data:

plate 1 %INH:

	Control	CMPD-1	CMPD-1	CMPD-2	CMPD-2	CMPD-3	CMPD-3	CMPD-4	CMPD-4	CNTRL CMPD	CNTRL CMPD	Control
	1	2	3	4	5	6	7	8	9	10	11	12
A	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value
B	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value
C	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value
D	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value
E	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value
F	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value
G	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value
H	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value	%INH value

IC50 Plots

Test Compounds

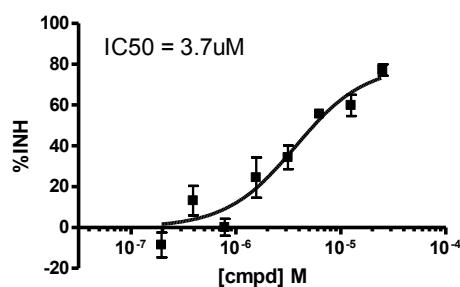### CMPD-1 – kinase 1

IC50 = 0.013uM

CMPD-2 – kinase 1

IC50 >10uM
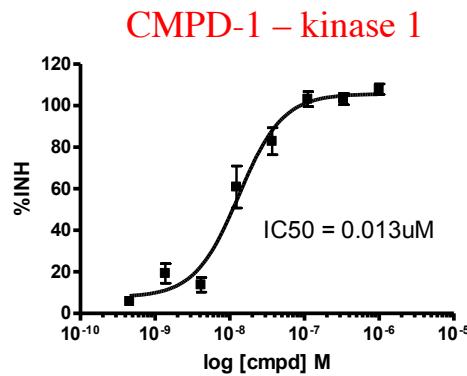
Sigmoidal dose-response (variable slope)
Best-fit values

BOTTOM	7.807
TOP	105.7
LOGEC50	-7.879
HILLSLOPE	1.474
EC50	1.321e-008

CMPD-3 – kinase 1

IC50 = 0.012uM
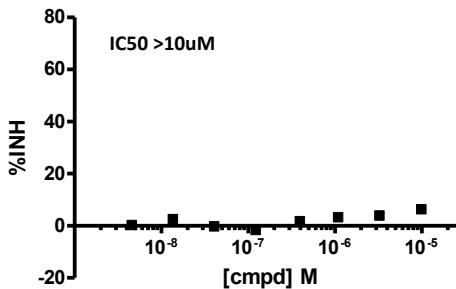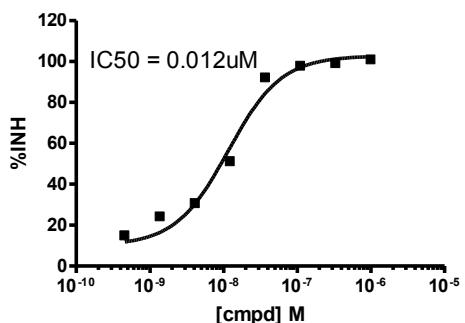
Sigmoidal dose-response (variable slope)
Best-fit values

BOTTOM	10.00
TOP	102.8
LOGEC50	-7.934
HILLSLOPE	1.212
EC50	1.163e-008

CMPD-4 – kinase 1

IC50 = 3.7uM

Sigmoidal dose-response (variable slope)
Best-fit values

BOTTOM	0.0
TOP	79.37
LOGEC50	-5.433
HILLSLOPE	1.318
EC50	3.694e-006

Control Compound(s):

CNTRL – kinase 1

IC50 = 0.027uM
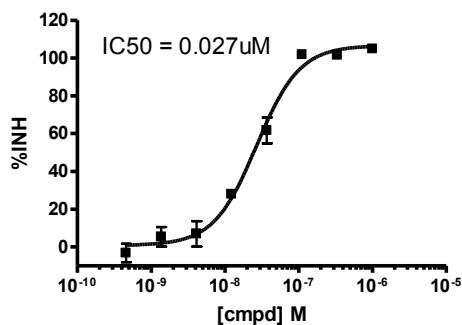
Sigmoidal dose-response (variable slope)
Best-fit values

BOTTOM	0.7413
TOP	106.7
LOGEC50	-7.569
HILLSLOPE	1.492
EC50	2.696e-008

IC50 summary:

Compound	kinase	kinase IC50 (uM)
CMPD-1	kinase-1	0.013
CMPD-2	kinase-1	>10
CMPD-3	kinase-1	0.012
CMPD-4	kinase-1	3.7
Control		**kinase IC50 (uM)**
CNTRL	kinase-1	0.027

Report Reviewed by:

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Deborah J. Moshinsky_____
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President_____
Title

DATE_____ __ __ _____
Date