

NanoBRET™ Intracellular Kinome-Wide Profiling of BTK inhibitors and Mechanism of Action Analysis of Selected Compounds.

Takeomi Inoue¹, Carolyn Foley², Adam Shutes², Rongmei Bao¹, Aki Emi¹, Tomohito Fujimoto¹, Yugo Narumi¹, Masaaki Sawa¹, James D. Vasta³, Matthew B. Roberts³, Yusuke Kawase¹
¹ Carna Biosciences, Inc., BMA, 1-5-5, Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan
² CarnaBio USA, Inc., 209 West Central Street, Suite 307, Natick, MA 01760, USA
³ Promega Corporation, 2800 Woods Hollow Road, Fitchburg, WI 53711, USA

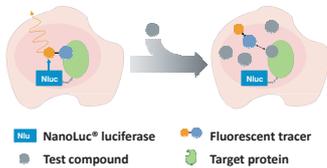


1. Overview

Since most kinase inhibitors target the structurally conserved ATP-binding site, it is important to thoroughly investigate the target selectivity of compounds. Kinase selectivity has been studied extensively using biochemical approaches in purified settings, but has rarely been studied in living cells. We report the utility of conducting the kinome-wide profiling in live HEK293 cells using the NanoBRET™ Target Engagement (TE) K192 Kinase Selectivity System [1], a format known as the NanoBRET™ TE Intracellular Kinase Assays.

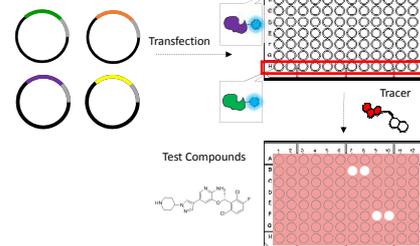
The intracellular kinase selectivity of 4 BTK inhibitors (Fenebrutinib, Ibrutinib, Dasatinib and Compound A) was evaluated. Based on the results and the knowledge that BTK inhibitors often target collateral TEC-family kinases, mechanism of action analyses were performed for selected compounds against BTK, TEC and BMX.

The principle of the NanoBRET™ TE Intracellular Kinase Assays



2. NanoBRET™ Intracellular Kinome-Wide Profiling

DNA Vector Set Encoding 192 Kinase/NanoLuc® Fusions



HEK293 cells were transiently transfected with 192 kinase-NanoLuc® fusion vectors and target occupancy of test compounds at 192 kinases was individually interrogated in living cells using only a single tracer, K-10. Each microwell row (encircled in red) contains a unique kinase/NanoLuc® fusion-expressing live cells.

3. Target List of NanoBRET™ Intracellular Kinome-Wide Profiling

AKT	CDK3	CLK	PKNO3
AKT1	CDK3A	CLK1	PKNO3A
AKT2	CDK3B	CLK2	PKNO3B
AKT3	CDK3C	CLK3	PKNO3C
AKT4	CDK3D	CLK4	PKNO3D
AKT5	CDK3E	CLK5	PKNO3E
AKT6	CDK3F	CLK6	PKNO3F
AKT7	CDK3G	CLK7	PKNO3G
AKT8	CDK3H	CLK8	PKNO3H
AKT9	CDK3I	CLK9	PKNO3I
AKT10	CDK3J	CLK10	PKNO3J
AKT11	CDK3K	CLK11	PKNO3K
AKT12	CDK3L	CLK12	PKNO3L
AKT13	CDK3M	CLK13	PKNO3M
AKT14	CDK3N	CLK14	PKNO3N
AKT15	CDK3O	CLK15	PKNO3O
AKT16	CDK3P	CLK16	PKNO3P
AKT17	CDK3Q	CLK17	PKNO3Q
AKT18	CDK3R	CLK18	PKNO3R
AKT19	CDK3S	CLK19	PKNO3S
AKT20	CDK3T	CLK20	PKNO3T
AKT21	CDK3U	CLK21	PKNO3U
AKT22	CDK3V	CLK22	PKNO3V
AKT23	CDK3W	CLK23	PKNO3W
AKT24	CDK3X	CLK24	PKNO3X
AKT25	CDK3Y	CLK25	PKNO3Y
AKT26	CDK3Z	CLK26	PKNO3Z
AKT27	CDK3AA	CLK27	PKNO3AA
AKT28	CDK3AB	CLK28	PKNO3AB
AKT29	CDK3AC	CLK29	PKNO3AC
AKT30	CDK3AD	CLK30	PKNO3AD
AKT31	CDK3AE	CLK31	PKNO3AE
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AKT35	CDK3AI	CLK35	PKNO3AI
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AKT37	CDK3AK	CLK37	PKNO3AK
AKT38	CDK3AL	CLK38	PKNO3AL
AKT39	CDK3AM	CLK39	PKNO3AM
AKT40	CDK3AN	CLK40	PKNO3AN
AKT41	CDK3AO	CLK41	PKNO3AO
AKT42	CDK3AP	CLK42	PKNO3AP
AKT43	CDK3AQ	CLK43	PKNO3AQ
AKT44	CDK3AR	CLK44	PKNO3AR
AKT45	CDK3AS	CLK45	PKNO3AS
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AKT48	CDK3AV	CLK48	PKNO3AV
AKT49	CDK3AW	CLK49	PKNO3AW
AKT50	CDK3AX	CLK50	PKNO3AX
AKT51	CDK3AY	CLK51	PKNO3AY
AKT52	CDK3AZ	CLK52	PKNO3AZ
AKT53	CDK3BA	CLK53	PKNO3BA
AKT54	CDK3BB	CLK54	PKNO3BB
AKT55	CDK3BC	CLK55	PKNO3BC
AKT56	CDK3BD	CLK56	PKNO3BD
AKT57	CDK3BE	CLK57	PKNO3BE
AKT58	CDK3BF	CLK58	PKNO3BF
AKT59	CDK3BG	CLK59	PKNO3BG
AKT60	CDK3BH	CLK60	PKNO3BH
AKT61	CDK3BI	CLK61	PKNO3BI
AKT62	CDK3BJ	CLK62	PKNO3BJ
AKT63	CDK3BK	CLK63	PKNO3BK
AKT64	CDK3BL	CLK64	PKNO3BL
AKT65	CDK3BM	CLK65	PKNO3BM
AKT66	CDK3BN	CLK66	PKNO3BN
AKT67	CDK3BO	CLK67	PKNO3BO
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AKT71	CDK3BS	CLK71	PKNO3BS
AKT72	CDK3BT	CLK72	PKNO3BT
AKT73	CDK3BU	CLK73	PKNO3BU
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AKT76	CDK3BX	CLK76	PKNO3BX
AKT77	CDK3BY	CLK77	PKNO3BY
AKT78	CDK3BZ	CLK78	PKNO3BZ
AKT79	CDK3CA	CLK79	PKNO3CA
AKT80	CDK3CB	CLK80	PKNO3CB
AKT81	CDK3CC	CLK81	PKNO3CC
AKT82	CDK3CD	CLK82	PKNO3CD
AKT83	CDK3CE	CLK83	PKNO3CE
AKT84	CDK3CF	CLK84	PKNO3CF
AKT85	CDK3CG	CLK85	PKNO3CG
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AKT88	CDK3CJ	CLK88	PKNO3CJ
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AKT91	CDK3CM	CLK91	PKNO3CM
AKT92	CDK3CN	CLK92	PKNO3CN
AKT93	CDK3CO	CLK93	PKNO3CO
AKT94	CDK3CP	CLK94	PKNO3CP
AKT95	CDK3CQ	CLK95	PKNO3CQ
AKT96	CDK3CR	CLK96	PKNO3CR
AKT97	CDK3CS	CLK97	PKNO3CS
AKT98	CDK3CT	CLK98	PKNO3CT
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AKT100	CDK3CV	CLK100	PKNO3CV
AKT101	CDK3CW	CLK101	PKNO3CW
AKT102	CDK3CX	CLK102	PKNO3CX
AKT103	CDK3CY	CLK103	PKNO3CY
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AKT106	CDK3DB	CLK106	PKNO3DB
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AKT108	CDK3DD	CLK108	PKNO3DD
AKT109	CDK3DE	CLK109	PKNO3DE
AKT110	CDK3DF	CLK110	PKNO3DF
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AKT216	CDK3HH	CLK216	PKNO3HH
AKT217	CDK3HI	CLK217	PKNO3HI
AKT218	CDK3HJ	CLK218	PKNO3HJ
AKT219	CDK3HK	CLK219	PKNO3HK
AKT220	CDK3HL	CLK220	PKNO3HL
AKT221	CDK3HM	CLK221	PKNO3HM
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AKT223	CDK3HO	CLK223	PKNO3HO
AKT224	CDK3HP	CLK224	PKNO3HP
AKT225	CDK3HQ	CLK225	PKNO3HQ
AKT226	CDK3HR	CLK226	PKNO3HR
AKT227	CDK3HS	CLK227	PKNO3HS
AKT228	CDK3HT	CLK228	PKNO3HT
AKT229	CDK3HU	CLK229	PKNO3HU
AKT230	CDK3HV	CLK230	PKNO3HV
AKT231	CDK3HW	CLK231	PKNO3HW
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