High Throughput Kinase Inhibitor Profiling in Live Cells with NanoBRET™ Target Engagement K192 Kinase Selectivity System

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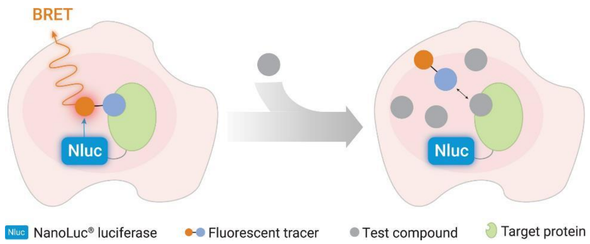
Abstract # 1292-A

1. Introduction

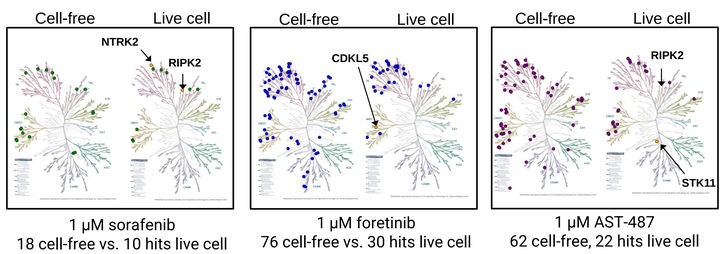
Kinase inhibitor profiling is an invaluable technique during the drug discovery process to understand inhibitor selectivity and determine off-target binding potential. We describe validation of an automated workflow using NanoBRET™ Target Engagement K192 Kinase Selectivity System for profiling compounds in live cells against 192 kinase targets. Transitioning assays from 96 to 384-well format required several optimization steps. For each kinase target, tracer concentration was re-optimized for automated acoustic dispensing into 384-well plates. With optimized tracer concentrations, we established control compound minimum significant ratio (MSR) by testing multiple biological replicates of full concentration response curves to evaluate assay and compound potency reproducibility. NanoBRET™ signal for each compound concentration was converted to % occupancy, and a % occupancy "cutoff value" was established to provide assay robustness guidance. The automated workflow presented is used by Promega's Tailored R&D Solutions group to provide researchers with a simple and reliable way to obtain live cell quantitative kinase inhibitor selectivity profiles.

2. NanoBRET™ Target Engagement Intracellular Kinase Assay

Compound engagement is measured in a competitive format through proximity-based energy transfer between NanoLuc® luciferase and a cell-permeable fluorescent NanoBRET™ tracer. Binding of the test compound results in a loss of NanoBRET™ signal between the target protein and the tracer inside intact cells.



3. Discover Novel Engagement Liabilities



Cell-free (1) and live cell K192 results were considered hits at ≥ 50% occupancy

[1]Davis, M., Hunt, J., Herrgard, S. *et al.* Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol* **29**, 1046–1051 (2011)

3. Integrated Automation System: 100 plates / day capacity

Robotic Arm: HighRes AC2 Robotic System

Transfection: Agilent Bravo Liquid Handler

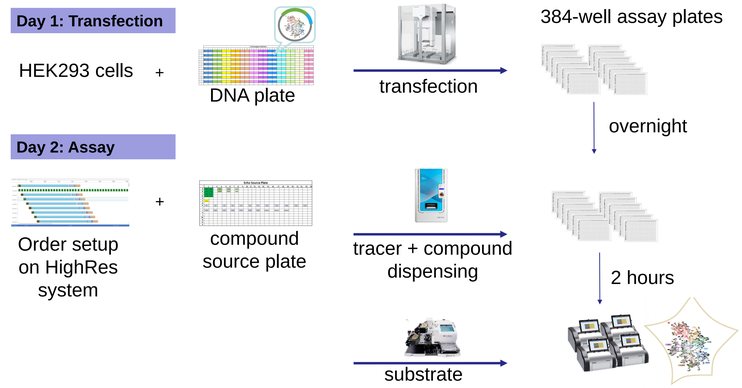
Tracer and compound dispensing:
- Echo 655T Acoustic Liquid Handler

System storage:
- Liconic ST-110 incubators
- HighRes Ambistore

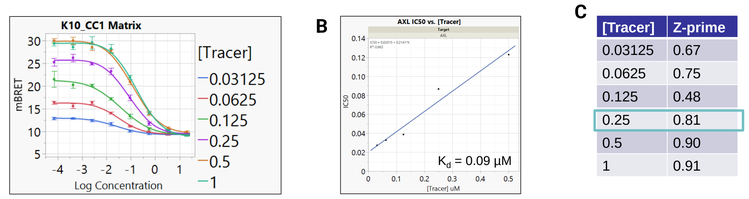
Substrate dispensing:
- BioTek MultiFloFX Dispenser

Plate Readers:
- GloMax Discover

5. High Throughput Automated Workflow



6. K192 Assay Validation in 384-well format

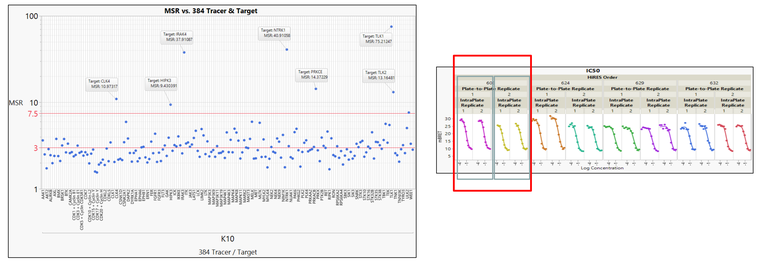


C

[Tracer]	Z-prime
0.03125	0.67
0.0625	0.75
0.125	0.48
0.25	0.81
0.5	0.90
1	0.91

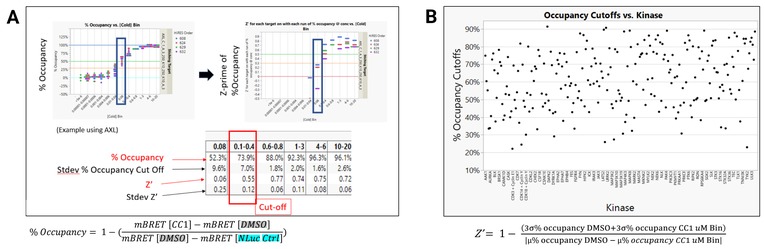
A) Impact of tracer concentration on test compound potency evaluated for each kinase target. AXL as example target is shown. **B)** Cheng-Prusoff linearized relationship of test compound (CC1) IC_{50} versus tracer concentration: $IC_{50} = K_i/K_d \times [Tracer] + K_i$. **C)** Z-prime to confirm assay quality at chosen concentration. Choosing tracer concentrations at or below K_d result in more accurate compound affinity estimates but with lower Z' value and assay window.

7. Tracking Individual Assay Performance



Run IC50 curves with control compound to evaluate assay variance
- Minimum 4 biological replicates
- Plate-to-plate (2) and within-plate replicates (2) in each biological replicate
- Calculate control compound (CC1) minimum significant ratio (MSR)
- Identify low performing assays and reoptimize

8. Building Confidence in Hit Calls



$$\%\ Occupancy = 1 - \left(\frac{mBRET\ [CC1] - mBRET\ [\boldsymbol{DMSO}]}{mBRET\ [\boldsymbol{DMSO}] - mBRET\ [\boldsymbol{NLuc\ Ctrl}]}\right)$$

$$Z' = 1 - \frac{(3\sigma\%\ \text{occupancy DMSO} + 3\sigma\%\ \text{occupancy CC1 uM Bin})}{|\mu\%\ \text{occupancy DMSO} - \mu\%\ \textit{occupancy CC1 uM Bin}|}$$

A) Calculated % occupancy values at different compound concentrations of CC1. Z-prime of % occupancy provides a measurement of how well the control is separated from the 0% occupancy control at a given compound concentration. Cutoff value is the lowest % occupancy where Z' – 1 Stdev Z' > 0

B) Occupancy cutoffs for kinase targets in K192 Kinase Selectivity System

9. Conclusions

K192 Kinase Selectivity System can be run using 384 well plates in automated high-throughput workflow
- Biological duplicates
- Raw data provided to clients through TRS Service at Promega

Validation experiments resulted in optimized assay parameters
- New optimized recommended tracer concentrations
- Control MSR for tracking individual assay performance over time and identifying assays that require additional optimization

% Occupancy values provide assay robustness guidance for single concentration selectivity profiling. K192 targets have a wide range of assay performance.
- Higher "cutoff" values indicate more variability at lower % occupancy values
- Highlights the need for following up with full concentration response curves for selectivity and potency confirmation

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