Live-Cell Real-Time Quantification of Drug-Target Engagement for Automation & High-Throughput Screening

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Abstract

Cell target engagement offers a powerful paradigm in drug discovery. It measures transitions in the thermodynamic state of a target protein as it engages with drug molecule. However, the current high-throughput strategies for cell target engagement are limited to end-point signals, poor utility across multiple challenging target families, and lack sufficient resolution for kinetic and mechanistic readouts. **We have developed a novel, highly sensitive and versatile fluorescence-based cell target engagement method that leverages complementation of split-RNase S**. This offers a unique combination of features, enabling highly sensitive detection of drug-target engagement in real-time systems. In addition, it integrates seamlessly with various applications designed for fluorescence detection, such as live-cell imaging.

This novel real-time cell target engagement method enables:

- Automation of scaled drug screening with fewer procedural steps;
- Improved signal-to-noise resolution advanced plate formats;
- Interrogation of challenging drug targets and and multiple modalities;
- Measurement of cellular binding affinities together with mechanistic insight.

Live-Cell Real-Time Drug-Target Engagement

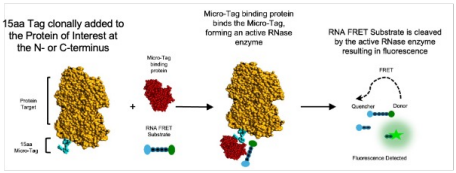
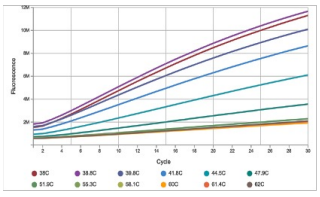


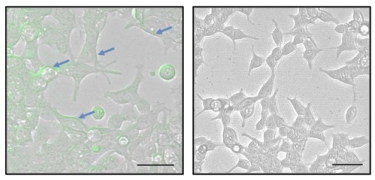
Diagram of the MICRO-TAG enzyme complementation system



FRET RNA substrate. Various options for donors and quenchers are depicted.

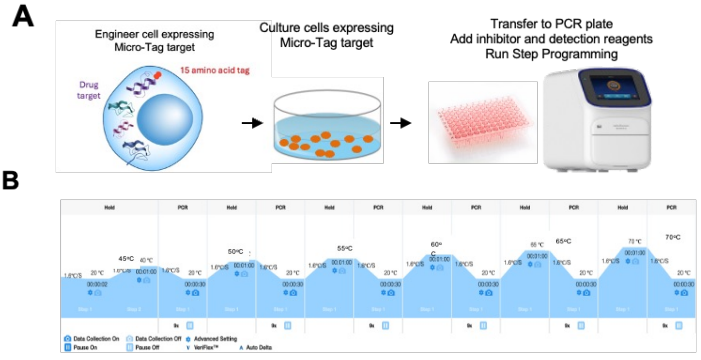


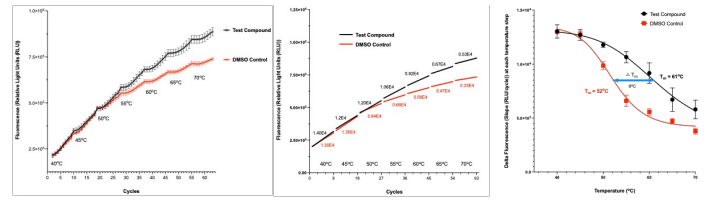
Teal-time curves from real-time system quantifying drug-stabilized target protein at various temperature points.



Real-time live-cell imaging of target. Transfected HEK293 cells are compared to untransfected control. Green represents areas with fluorescence of target-induced enzyme complementation.

Step Gradient Method Streamlines In-Cell Drug-Target Engagement



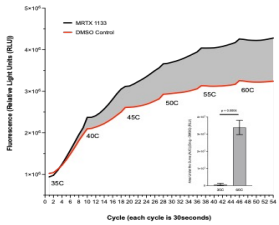


Step gradient system for streamlined in-cell drug-target engagement

(A) Schematic of the streamlined workflow for step-gradient cell target engagement.
(B) Programming of real-time qPCR instrument for use with MICRO-TAG in step gradient. Temperatures, durations and number of cycles used under each Hold and detection step are shown.
(C) Representative readout from real-time system measured S-Crizotinib binding and stabilizing MTH1. Step-gradient profile of DMSO control is plotted together with S-Crizotinib.
(D) Linear regression analysis of each detection step at specific temperatures from (C). Slopes are indicated above each temperature point.
(E) Delta fluorescence analysis of data from (D). Melting Temperature (T_m) values extracted from each curve together with delta-T are indicated.

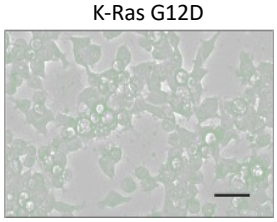
Validation of K-Ras Inhibitor MRTX1133 with MICRO-TAG Real-Time Step Gradient

Step gradient real-time drug-target engagement



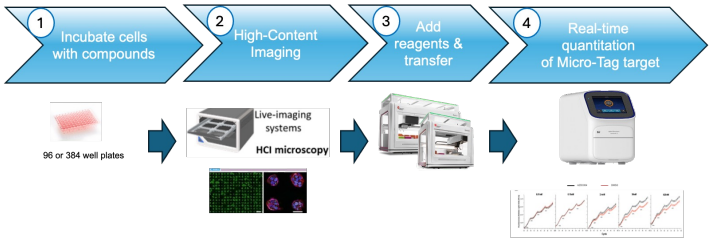
Step-gradient curves from real-time monitoring of K-Ras (G12D) with 2.5uM MRTX-1133 and DMSO control. The inner histogram shows the AUC after 35C heating step compared with AUC after 60C heating step.

Live–cell imaging of K-Ras using MICRO-TAG system



Real-time live-cell imaging of Micro-Tag K-Ras (G12D). Green represents areas with fluorescence of target-induced enzyme complementation.

Automation & High-Throughput Screening



MICRO-TAG step-gradient method merged with real-time systems offers a versatile, scalable, cost-efficient and robust strategy for evaluation of cellular drug-target engagement. The method is suited for challenging drug targets that cannot be expressed outside the cellular context. The real-time strategy empowers the assay setup and analysis through increased sensitivity and flexibility of instrumentation programming and choices in donor/quencher FRET pairs.

This methodology streamlines drug discovery processes offering savings on time and cost, allowing for improved throughput and ease of automation, while enabling discovery of therapeutics for challenging drug targets.

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