REAL-TIME CELL TARGET ENGAGEMENT

MICRO-TAG PLATFORM

White paper v1.

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CELL TARGET ENGAGEMENT

Analysis and confirmation of direct binding of a drug to its intended target is a critical part of drug discovery. However, most proteins that are considered therapeutic targets are not amenable to gold standard target engagement biophysical methods or co-crystallization strategies due to the artificial acellular environment employed by these methods. Challenging drug targets require the complexities of the physiological cellular environment for proper folding and function. In-cell drug target engagement offers a unique means for validating and analyzing direct drug-target interaction in the context of living cells.

Micro-Tag fluorescence-based cell target engagement technology is based on an enzymatic reaction for fluorescence development and uses highly sensitive real-time instruments, providing better signal to noise and improved sensitivity and dynamic range than other similar strategies. An enzymatic reaction generating a fluorescent signal amplifies the read-out allowing for detection of lower levels of protein resulting in higher dynamic range. Fluorescence based readouts have the advantage of integrating with highly sensitive instrumentation for fluorescence detection. In addition, the detection chemistry of Micro-Tag technology offers versatility in choices of fluorophore/fluorescence wavelengths allowing for multiplexing capability with other florescence-based techniques.

MICRO-TAG PLATFORM

Micro-Tag technology is based on enzyme complementation with a fluorescent readout. It involves a short 15 amino acid cloned to either N- or C-terminus of a target protein of interest. The Micro-Tag is predominantly hydrophilic making it a better tag for use in thermal shift assays than other similar enzyme complementation tags that are predominantly hydrophobic requiring addition of a spacer/linker region to extend the tag from the protein target. The Micro-Tag is one subunit of a two-subunit enzyme. Binding of the Micro-Tag to the large subunit forms an active RNase enzyme capable of cleaving a specific RNA sequence (**Figure 1**). In this target engagement assay the Micro-Tag in complex with its binding partner cleaves a specific RNA FRET (Förster or Fluorescence Resonance Energy Transfer) substrate to generate fluorescence signal. The FRET substrate offers versatility through choice of different donor/quencher FRET pairs to accommodate different wavelength detection. Generating fluorescence by an enzymatic reaction amplifies the signal making the system highly sensitive with a wide dynamic range.

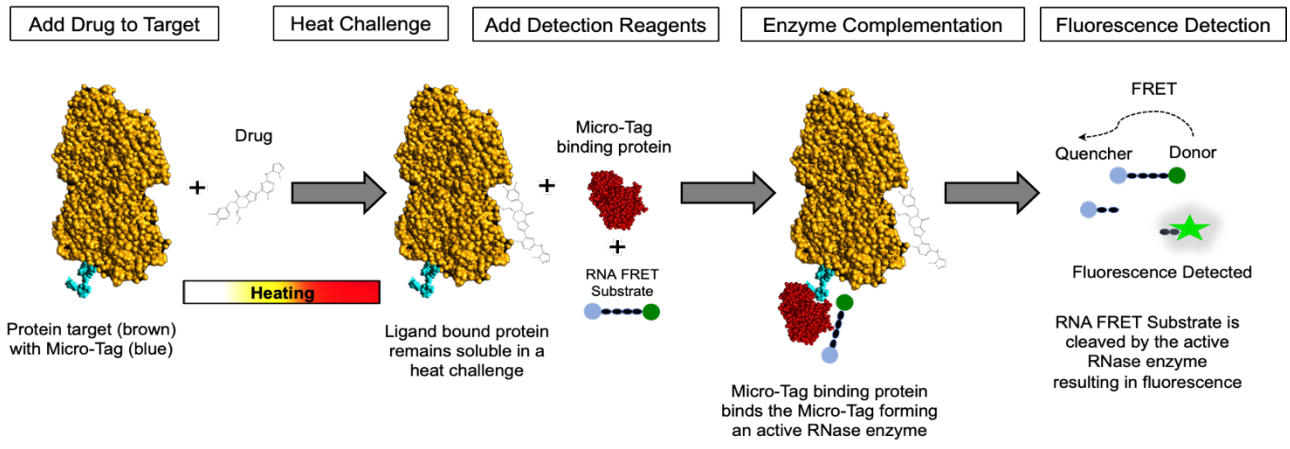

Figure 1. Micro-Tag enzyme complementation system for cell target engagement.

REAL-TIME MONITORING OF DRUG-TARGET ENGAGEMENT

Micro-Tag cell target engagement technology offers two strategies for determining drug protein binding: **(1)** conventional target engagement involving the initial identification of the temperature of aggregation or melting of the protein of interest, followed by screening for ligand binding at that specific temperature, or **(2)** **Real-time cell target engagement**, which employs a step gradient method that utilizes the thermal cycler programming capability of highly sensitive real-time systems (**Figure 2**). Micro-Tag technology improves upon previous cell target engagement techniques through increased sensitivity and versatility of the detection chemistry, multiplexing capability, decreased time performing the experiments, significant cost savings from streamlined workflow, and integration with standard cell monitoring instrumentation.

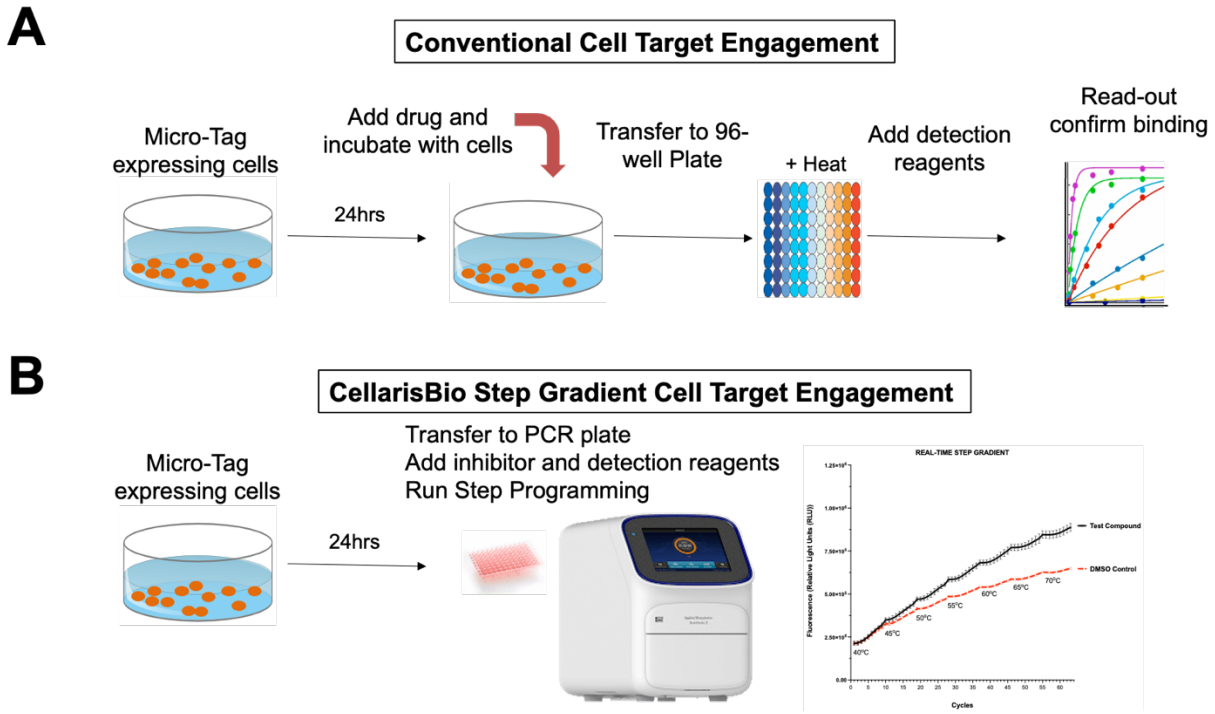

Figure 2. Cellular target engagement strategies employing protein thermal shift for drug discovery.

(A) Conventional thermal shift assay for cell target engagement.
(B) Micro-Tag real-time Step gradient cell target engagement assay employing ThermoFisher QuantStudio real-time instrument.

Testing ligand binding using the Step gradient thermal cycling program offers convenience by decreasing the number of processing steps involved in setting up the reaction. In addition, this “mix and detect” feature of the protocol without any pre-incubation allows for faster turnaround time and provides a real-time analysis of drug target interaction within a cellular context prior to any equilibrium reached between drug and target. This provides additional layers of information regarding rate of drug association with target when the target exits within its native cellular environment.

POWER OF MICRO-TAG PLATFORM

Advantages of Micro-Tag cell target engagement technology:

- Saves time and cost; no need to perform thermal profiling of the target prior to ligand testing. Therefore, less processing steps streamlines workflow, uses less reagents, and faster time from setup to analysis of binding.
- Increased sensitivity with greater dynamic range with real-time instruments.
- Increased flexibility of the assay. The assay is not limited to just one substrate. Choice of multiple FRET pairs and substrate for modified readout offers versatility in experimental setup.
- Increased throughput and ease of automation. The technique is amenable to the 384-well system of real-time instruments.
- Less troubleshooting in this assay from fewer procedural steps. Some targets melting temperature can be a technical challenge. However, the Step gradient technique provides detailed information of binding across all temperatures in one run.
- Technical support with assay optimization: Micro-Tag system supports assay optimization and technology transfer/licensing.

The Micro-Tag Step gradient assay for in-cell drug-target engagement merged with ThermoFisher's QuantStudio real-time instrument offers a versatile, faster, cost-efficient, and more robust strategy for analyzing the binding of a drug to its target within the physiological environment of a cell. This technology is applicable to challenging protein 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 offered with the substrate. This methodology streamlines the drug discovery process offering savings on time, cost, allows increased throughput and ease of automation while enabling discovery of therapeutics for challenging drug targets.

Some of the drug targets we worked with: