CellarisBio

# PRODUCT WHITE PAPER

## MICRO-TAG® Real-Time Cellular Target Engagement Kit


**Product Number:** RT-CTE-100

## MICRO-TAG® Technology Overview

MICRO-TAG® is a novel fluorescence-based cellular target engagement technology built on Split RNase S enzyme complementation. A short, minimally perturbing peptide tag fused to the target protein enables conditional enzyme reconstitution only when the target remains folded and soluble following thermal challenge. Upon complementation with the large subunit, enzymatic cleavage of a RNA substrate amplifies signal, enabling sensitive, quantitative detection of target engagement directly in a cellular context.

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## Product in Snapshot

**MICRO-TAG® Real-Time Cellular Target Engagement Kit enables:**

- Temperature-series measurement of cellular target engagement
- Quantification of target engagement across multiple thermal states
- Fluorescence-based detection on existing real-time instruments (e.g., QuantStudio)

**Designed specifically for:**

- Challenging and intrinsically disordered drug targets
- Targets not amenable to purification or classical biophysical assays

**Core value:**

- Removes dependence on a single Tagg50 point
- Provide real-time sensitivity to cellular target engagement
- Captures multi-state and temperature-dependent engagement behavior
- Scales to screening and mechanistic workflows

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## What Makes This a Temperature-Series System

**This kit is fundamentally built around:**

- Programmable **step-gradient temperature interrogation**
- Measurement of engagement **across a defined thermal range**
- Simultaneous assessment of aggregation behavior and ligand stabilization

**Why this matters:**

- Many challenging targets exhibit engagement only in specific temperature windows
- Single-temperature assays introduce bias and variability
- Real-time temperature-series analysis improves robustness, sensitivity and interpretability

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## Enzyme Complementation Chemistry and Signal

**Detection chemistry:**

- Split RNase S enzyme complementation
- 15-aa hydrophilic S-tag fused to the target protein
- Designed to minimize structural perturbation

**Thermal behavior:**

- Folded target protein → tag accessible → enzyme complements → high fluorescence signal
- Denatured/aggregated protein → tag buried → no complementation → low fluorescence signal

**Signal generation:**

- Active RNase is complemented (15-aa small subunit + large subunit)
- Active RNase cleaves RNA FRET substrate
- Enzymatic amplification yields high signal-to-noise fluorescence
- Multiple donor–quencher FRET pairs supported for various signal detection spectra

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## Step-Gradient Temperature-Series Workflow

**Workflow executed directly on real-time instruments:**

1. Compound incubation with intact cells or non-denaturing lysates
2. Programmable stepwise temperature escalation
3. At each step: thermal perturbation → return to permissive temperature
4. Fluorescence signal generated and recorded
5. Engagement mapped across the full temperature series

**Key advantages:**

- No need to pre-determine melting or aggregation temperature (Tagg)

- Reduced setup time and fewer failure points
- Engagement and stability assessed in a single run

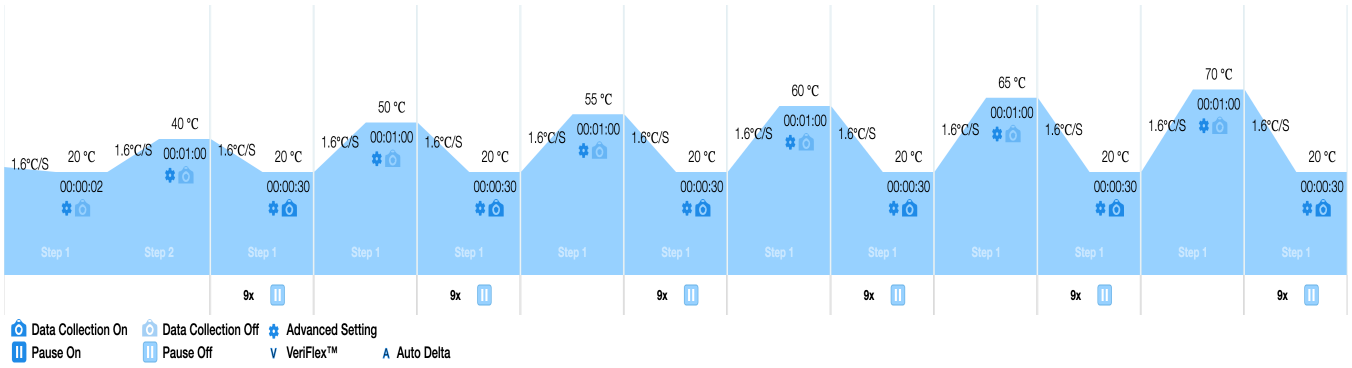


**Figure 1.** Step-gradient temperature-series workflow for real-time cellular target engagement. Cells or non-denaturing lysates are subjected to a programmable temperature series, followed by fluorescence-based detection at each thermal step, enabling measurement of ligand-dependent target stabilization across multiple thermal states.

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## Real-Time Detection on Existing Instrumentation

**Compatible platforms include:**

- Applied Biosystems QuantStudio™ real-time systems
- Other comparable real-time PCR instruments with programmable thermal control and fluorescence detection

**Practical benefits:**

- No specialized biophysical hardware required
- 96- and 384-well compatibility
- Automation- and HTS-ready workflows

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## Quantitative Outputs

**Supported readouts include:**

- Fluorescence Area Under Curve (AUC) across the temperature range
- Slope-based signal accumulation metrics
- Single-dose or dose–response formats

- EC50 determination from temperature-series data

**Why temperature-series metrics matter:**

- More robust than single-point measurements
- Better signal resolution for conformationally dynamic targets
- Improved discrimination between compound classes

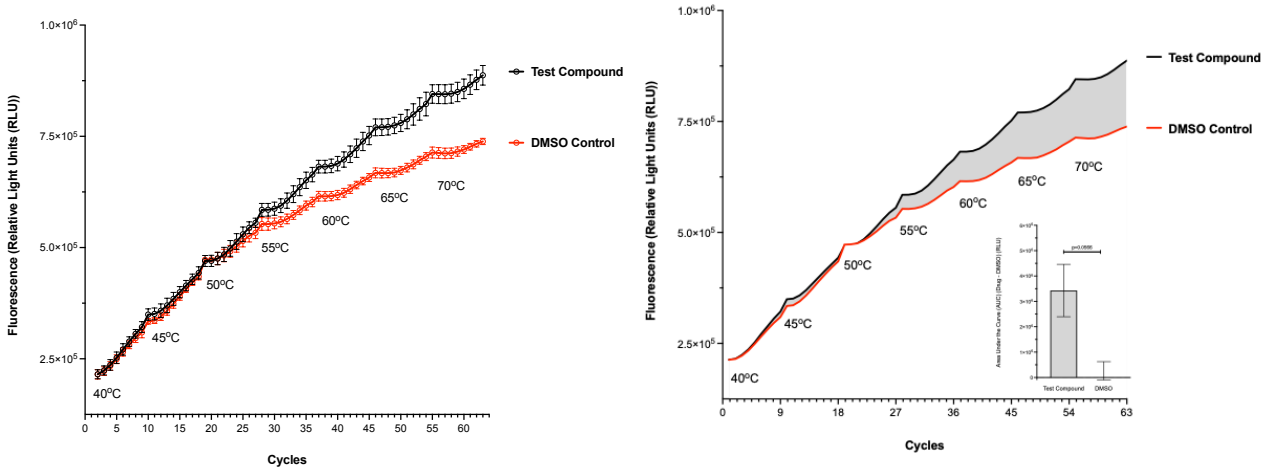


**Figure 2.** Representative temperature-series cellular target engagement profiles. Fluorescence signal measured across a thermal range reveals ligand-dependent stabilization of the target protein (left), supporting quantitative analysis using aggregated temperature-series metrics (right).

## Therapeutic Modalities Supported

**Compatible with most small-molecule modalities, including:**

- Reversible inhibitors
- Covalent inhibitors
- Allosteric modulators
- Molecular glues
- Targeted protein degraders (PROTACs and related bifunctionals)
- Stabilizers and neo-interaction inducers
- Fragment-derived and chemically diverse small molecules

## Target Classes Supported

The MICRO-TAG® Real-Time CTE Kit is specifically tuned for challenging and intrinsically disordered targets, including:

- Transcription factors (e.g., β-catenin, MYC, STAT family)
- Membrane proteins (e.g., GPCRs and other multi-pass receptors)
- Kinases and pseudo-kinases (e.g., KRAS and signaling-associated kinases)
- Structural and scaffolding proteins
- Chromatin-associated and multiprotein complex components
- Other non-traditional or difficult-to-purify protein classes

## Kit Components

**Included:**

- Real-time–optimized RNA FRET substrate (200µl)
- S-protein enzyme complementation reagent (200µl)
- Assay buffers (10ml)
- Positive control target plasmid (10µg)
- Inhibitor for positive control target (10µl, 10mM DMSO stock)

**Not included:**

- Drug target construct (ordered separate from CellarisBio website)

**Expandable:**

- Custom construct design for user-defined protein pairs
- Stable cell line generation available

## Target Selection and Expandability

**Target strategy:**

- Target constructs ordered separately in plasmid format
- 100+ validated off-the-shelf targets available
- Custom constructs available for any desired target
- Compatible with transient expression and stable cell lines

**Applications**

- Hit discovery (conventional plated screens of DNA-Encoded Libraries)
- Hit validation (after conventional plated screens of DNA-Encoded Libraries)
- Mechanism-of-action studies
- Comparative profiling across compound classes
- Advanced discovery workflows for challenging targets

## Summary

The MICRO-TAG® Real-Time Cellular Target Engagement Kit delivers fluorescence-based, temperature-series quantification of drug–target engagement using programmable real-time instruments. By enabling multi-temperature interrogation in intact cellular systems, the kit provides robust, sensitive, and mechanistically informative engagement data for challenging and intrinsically disordered drug targets across all small-molecule modalities.

## Protocols

www.cellarisbio.com/protocols

## References

1. Babic et al. *Real-Time Cellular Target Engagement Using MICRO-TAG Technology.* **SLAS Discovery**. 2026.