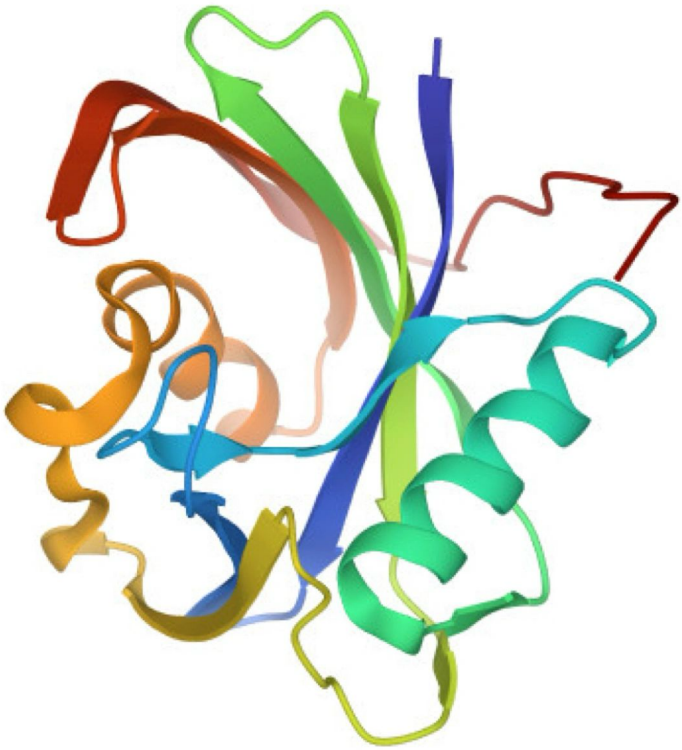
MTH1

MutT Homolog 1 (Homo sapiens)



Case study

V2.0

About MTH1

The human MutT Homolog1 (MTH1) protein encoded by *NUDT1* gene is a DNA repair enzyme that eliminates 8-oxo-7,8-dihydro-2'-deoxyguanosine triphosphate (8-oxodGTP) through its pyrophosphatase activity. The enzyme hydrolyses oxidized purines and prevents their addition onto the DNA chain.

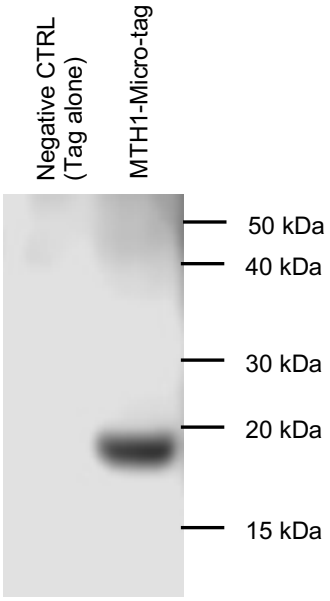
MTH1 has an important role in aging and cancer development. The overexpression and crucial role of MTH1 have been verified for a broad spectrum of tumors, such as renal-cell carcinoma, brain tumors, primary non-small cell lung tumors, colorectal cancer, non-small cell lung cancer, gliomas, breast cancer, myeloma, and squamous cell carcinoma. Several studies have identified MTH1 inhibition as an effective tumor-suppressive strategy. The indispensability of MTH1 for cancer cell growth or survival under oxidative conditions is an ongoing study to clearly understand cellular response to MTH1 inhibition.

Therapeutic challenges of MTH1

The crystal structure of MTH1 has been reported and several compounds have been identified that bind to the active site of MTH1 and inhibit its activity.

The (S)-enantiomer of crizotinib, a well-known Met/ALK inhibitor, was first-in-class inhibitor of MTH1 reported in 2014 as a nanomolar suppressor of MTH1 activity. By contrast, the (R)-enantiomer only inhibited MTH1 catalytic activity in the micromolar range. Several studies have shown (S)-crizotinib treatment leads to elevated levels of 8-oxodGTP. Treatment with (S)-crizotinib, but not (R)-crizotinib, reduced xenograft tumor formation, demonstrating that inhibition of MTH1 activity has potential as a therapeutic strategy.

Figure 1: Step 1A - Expression test of MTH1 in HEK293 cells.



Blotted with anti-Micro-tag antibody

Targeting MTH1 using MICRO-TAG system

Cellular target engagement has been instrumental in demonstrating selectivity of novel MTH1 inhibitors, especially Astra Zeneca's TH287 and TH588. More potent and selective anti-MTH1 inhibitors are desired in order to target a variety of cancers in single-agent and combination therapies.

CellarisBio's MICRO-TAG cellular target engagement technology offers a unique way to approach to identify novel therapeutic candidates that directly engage with MTH1 within the context of the cell.

The MICRO-TAG system designed to identify direct binders to MTH1 has 4 steps:

Step 1: Design a MICRO-TAG reporter for the target.

Step 2: Establish thermal melting profile of the target.

Step 3: Run a pilot test with a reference compound.

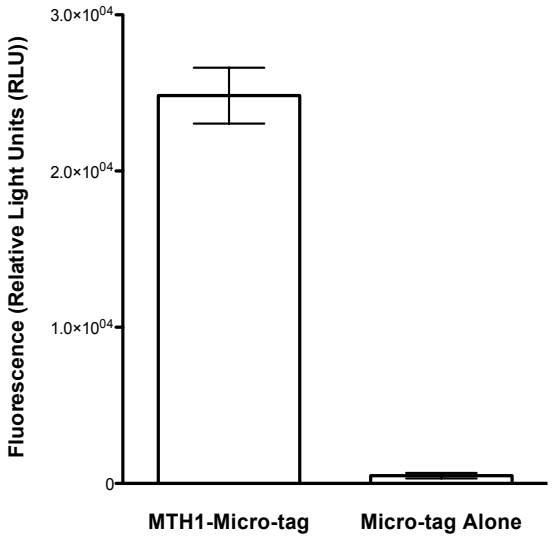
Step 4: Scale the system for compound discovery.

Step 1: Design MICRO-TAG reporter system specific for MTH1

DNA construct encoding for MTH1 transfected into HEK293 cells and expression of the target was detected, as shown in **Figure 1**.

MICRO-TAG system works on the basis of enzyme complementation that generates fluorescence signal. Hence, following satisfactory expression of the target, enzyme complementation of the MICRO-TAG construct is tested, as shown in **Figure 2**.

Figure 2: Step 1B - Activity of MICRO-TAG target construct for MTH1.



Step 2: Establish the thermal melting profile

Once target expression and enzyme complementation steps are completed, the target in the MICRO-TAG system is tested for thermal profile. Intact HEK293 cells carrying the MTH1 expression construct are subjected to short thermal gradient ranging typically from 37C to 75C.

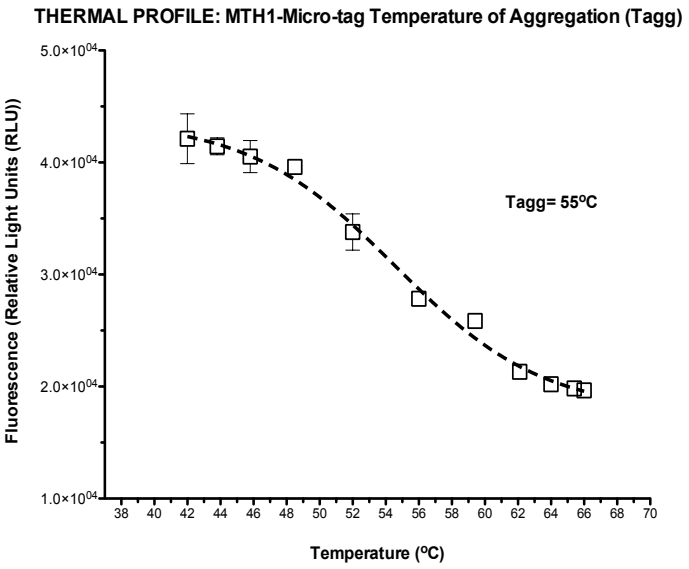
Fluorescence signal originating from MTH1 is detected at each temperature point is detected and thermal melting profile of the target is established. This step has to be performed empirically in order to establish the unique fingerprint of the target. The temperature of aggregation (T_{agg}) for MTH1 is calculated as 55C, as shown in **Figure 3**. At this temperature, the target in the cell is expected to be in half structured and half unwound state.

Step 3: Run a pilot test with a reference compound

Once the thermal melting profile of the target is empirically established, response of the target can be tested in a pilot test using a reference or a tool compound. Reference compounds specific to the target are preferred.

To test response of MTH1, we used the reference compound S-Crizotinib. Intact cells carrying MICRO-TAG constructs for MTH1 were treated with S-Crizotinib in dose-dependent manner, then exposed to thermal challenge at 55C. The ligand stabilized the target in dose-dependent manner with EC50 of 58nM, as shown in **Figure 4**.

Figure 3: Step 2 – Thermal melting profile of MTH1.



The pilot phase also provides an opportunity to optimize the assay to capture more response from the cellular drug-target engagement.

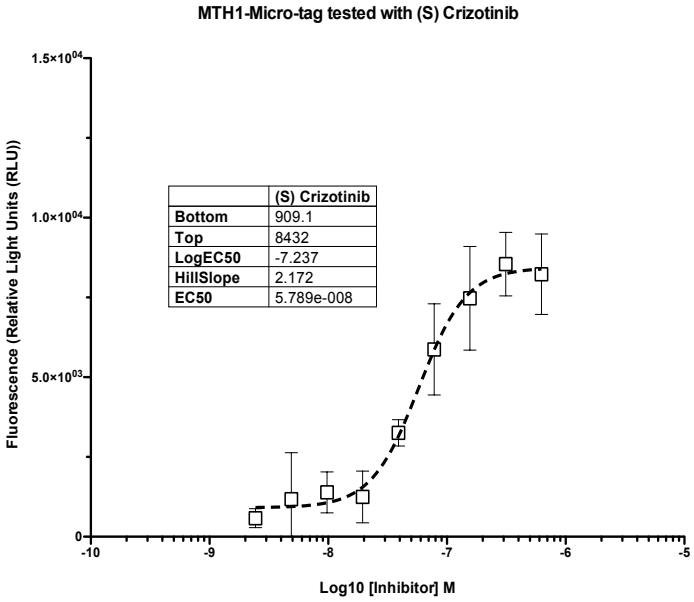
Step 4: Scale the system for compound discovery

Once the pilot step is accomplished, the reporter assay is ready for scale-up. Depending on requirements, there are several options to consider.

Transient expression of the MICRO-TAG target can be used for low-throughput screens aiming at validation of advanced drug candidates. Stable MICRO-TAG reporter assay can be generated using lentiviral knock-in or CRISPR approach. This latter approach is more tuned for high-throughput primary screens aimed at discovering of novel candidates.

In the case of MTH1, we proceeded with transient-expression system and set up a medium-throughput screen for validation of already identified small molecule candidates.

Figure 4: Step 3 – Cell target engagement of reference compound S-Crizotinib with MTH1.



Accelerate and De-Risk Drug Discovery with CellarisBio

CellarisBio's is a drug discovery technology company, based in San Diego, California. Our MICRO-TAG cell target engagement platform is built to tackle challenging drug targets.

We work across various therapeutic targets classes such as:

- Enzymes
- Membrane proteins
- Transcription factors
- Other challenging proteins

We work with multiple therapeutic modalities such as:

- Small molecules
- Peptide
- PROTACS
- Antibodies

Accelerate your drug discovery with CellarisBio:

- Discover drug candidates using DNA-Encoded Libraries or plated libraries,
- Validate drug candidates for potency and selectivity,
- Analyze for in-cell kinetics and live-cell imaging.

Some of the drug targets we worked with:

What drug targets are you interested in?

San Diego, California
www.CellarisBio.com

hi@cellarisbio.com