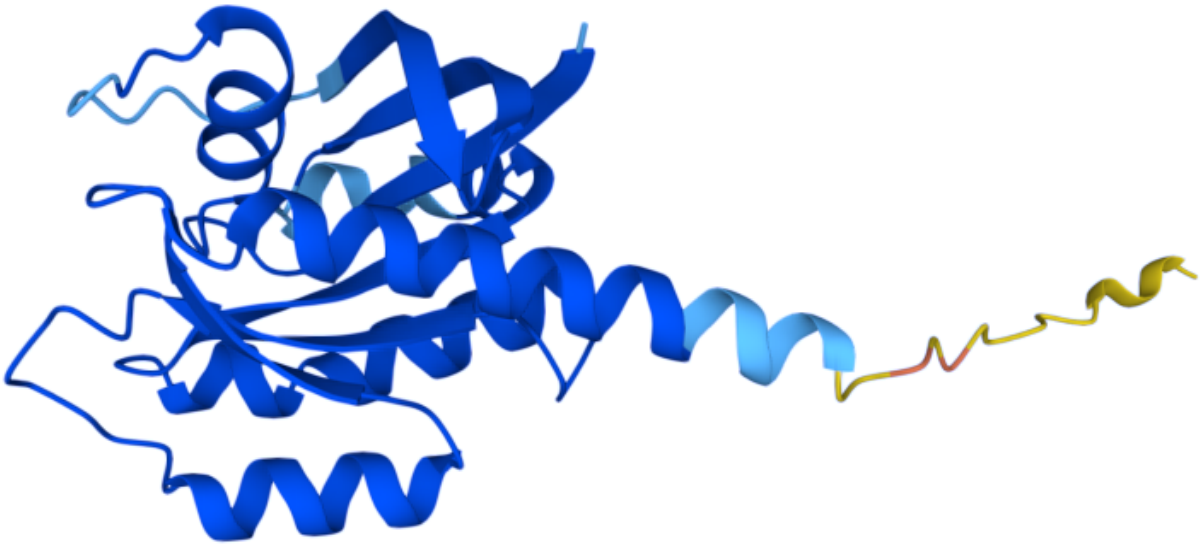
KRAS

GTPase KRas (Homo sapiens)



Case study

V2.0

About KRAS

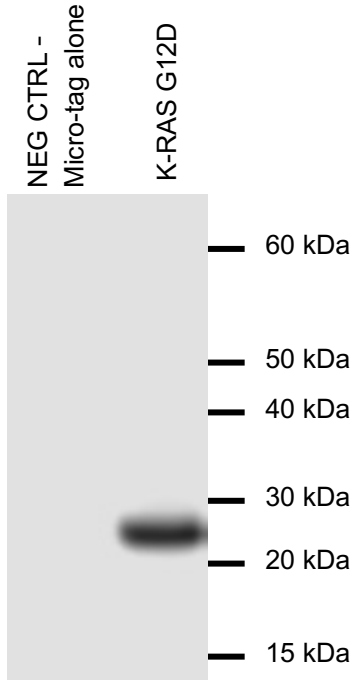
KRAS is a signal transducer protein that plays a significant role in cell signaling and the regulation of cell proliferation. KRAS is a gene that codes for the GTPase KRas, a member of the Ras protein family, and it is closely related to isoforms HRAS and NRAS. Ras proteins are a set of oncoproteins that regulate the Ras/Raf/MAPK pathway, and KRAS is the most oncogenic of the isoforms. Ras proteins bind GDP/GTP and have low intrinsic GTPase activity. Their activity alternates between an inactive form bound to GPD an active form bound to GTP.

KRAS activating mutations are known to be associated with a variety of human cancers such as acute myelogenous leukemia (AML) and gastric cancer, as well as developmental disorders like Noonan syndrome, cardio-facio-cutaneous syndrome (CFCS), and Costello syndrome.

Therapeutic challenges of KRAS

KRAS is a structurally well-defined enzyme of approx 25 kDa in size. The G domain of KRAS has over 150 amino acids arranged in six beta strands and five alpha helices. The G domain is joined by a hypervariable region and two switch regions, switch I and switch II. The GTP-converting active site of KRAS is well conserved.

Figure 1: Step 1A - Expression test of KRAS G12D in HEK293 cells.



Blotted with anti-Micro-tag antibody

Targeting KRAS with MICRO-TAG target engagement

KRAS has long been considered an undruggable target due to its small size and relatively smooth surfaces. There are now several clinical candidate drugs targeting KRAS. More potent and selective anti-KRAS 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 KRAS within the context of the cell.

The MICRO-TAG system designed to identify direct binders to KRAS G12D has 4 steps:

Step 1: Design a MICRO-TAG reporter for the target.
Step 2: Establish thermal melting profile of the target.

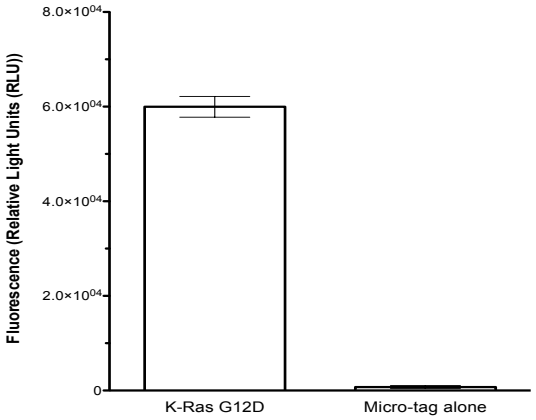
Step 3: Run a pilot test with a reference compound.
Step 4: Scale the system for compound discovery.

Step 1: Design MICRO-TAG reporter system specific for KRAS G12D

DNA construct encoding for KRAS G12D 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 KRAS G12D.



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 expression construct are subjected to short thermal gradient ranging typically from 37C to 75C.

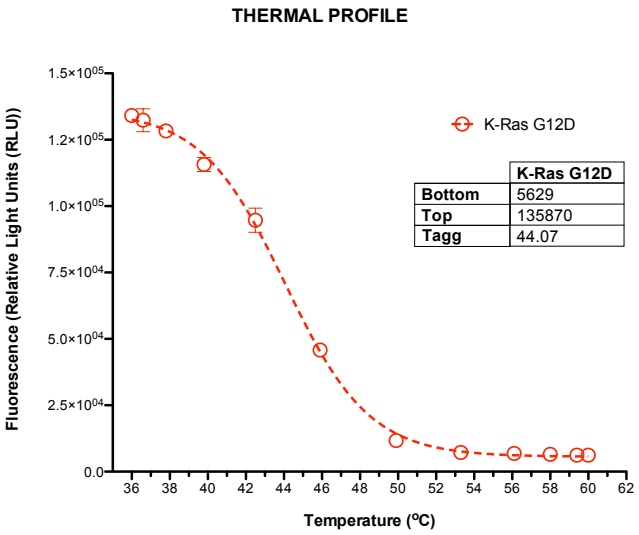
Fluorescence signal originating from KRAS G12D is detected at each temperature point is detected and thermal melting profile of the target is established. This step has to be performed empirically in order to establish the unique fingerprint of the target. The temperature of aggregation (T_{agg}) for KRAS G12D is calculated as 44C, 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 KRAS G12D, we used the reference compound MRTX1133. Intact cells carrying MICRO-TAG constructs for KRAS G12D were treated with MRTX1133 in dose-dependent manner, then exposed to thermal challenge at 44C. The ligand stabilized the target in dose-dependent manner with EC50 of 36nM, as shown in **Figure 4**.

Figure 3: Step 2 – Thermal melting profile of KRAS G12D.



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

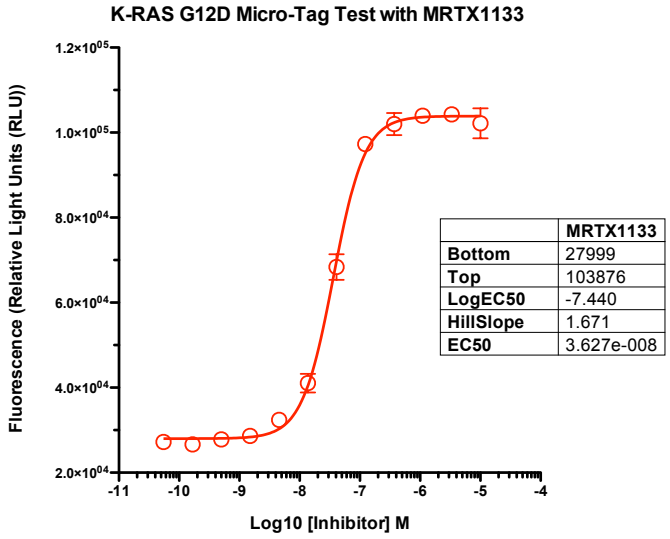
Step 4: Scale the system for compound discovery

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 KRAS G12D, 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 MRTX1133 with KRAS G12D.



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?

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