APPLICATION NOTE

KRAS GTP competitors and KRAS/SOS1 inhibitors screening with new HTRF biochemical-based assays.

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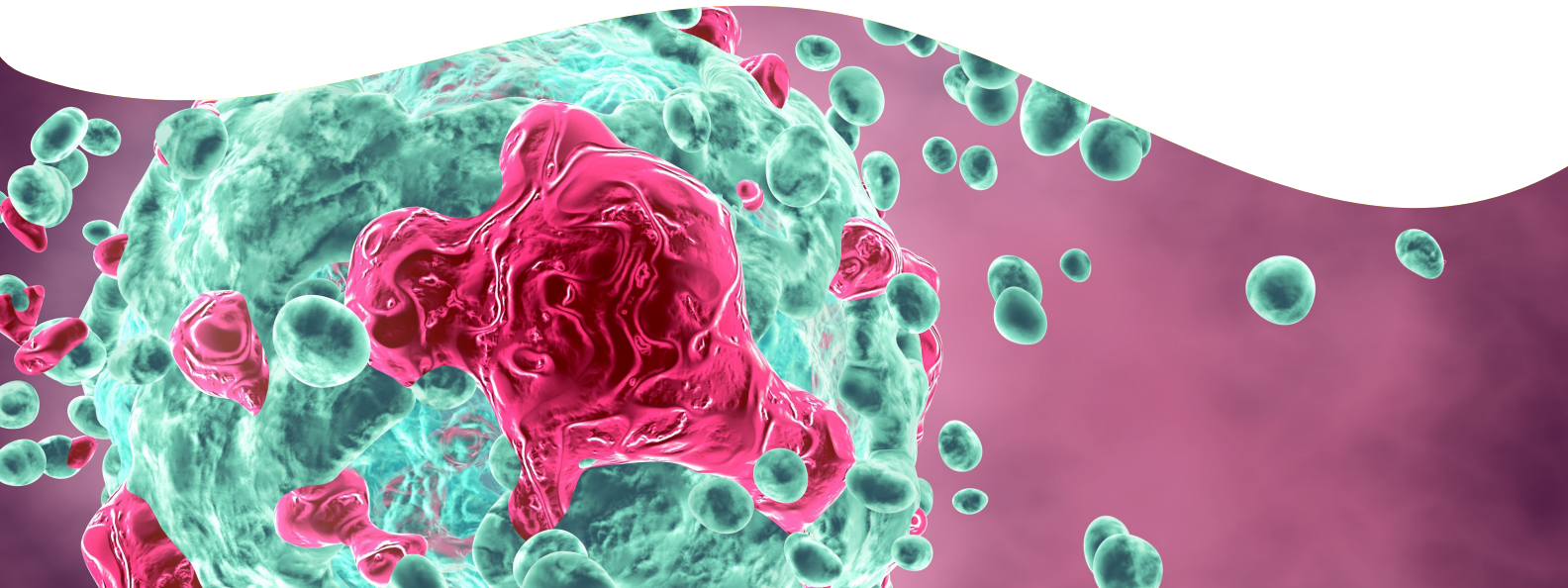
KRAS is a small GTPase implicated in various biological processes such as cell proliferation, cell survival and cell metabolism. This proto-oncogene is well known to be mutated in many cancer subtypes, inducing in uncontrolled proliferation and cell metabolism modifications. It thereby contributes to the Warburg effect in cancer cells. Like the majority of small GTPase, KRAS binds to GDP in its inactive form, or it binds to GTP to switch into the active form. KRAS G12C is one of the most commonly represented mutant forms in cancer, which leads to a permanently active state of KRAS. The Ras guanine nucleotide exchange factor, also called SOS1, is a GEF protein promoting the active form of KRAS. The upregulation of the KRAS/SOS1 interaction leads to cancer phenotypes.

Identifying new KRAS/GTP competitors and KRAS/SOS1 inhibitors is therefore a relevant strategy to control biological processes involved in cancer growth by reducing KRAS activity and associated downstream signaling pathways.

The KRAS WT and G12C GTP binding kits (Revvity #64BDKRASWPEG/H ; #64BDKRASGPEG/H) and KRAS WT and G12C/SOS1 binding kits (Revvity #64KRASWTPEG/H; #64KRASG12PEG/H) are novel assays using HTRF® technology, a robust and homogeneous TR-FRET platform. These assays enable the identification of all GTP competitors and KRAS/SOS1 inhibitors in a biochemical format utilizing recombinant KRAS and SOS1 human tagged proteins.

This application note provides a convincing demonstration of the reliability of the HTRF KRAS portfolio to identify and classify chemical compounds, such as KRAS GTP competitors or KRAS/SOS1 inhibitors, in a biochemical context.



Assay Workflow

Principle of the HTRF KRAS assays:

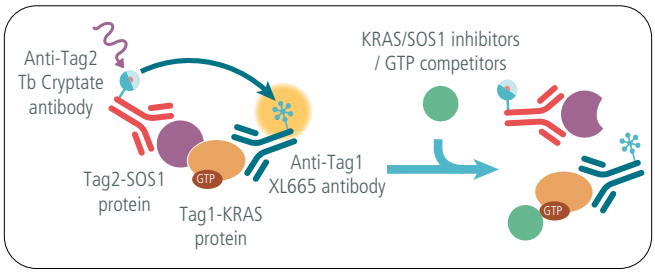


Figure 1: Principle of the KRAS/SOS1 binding assays

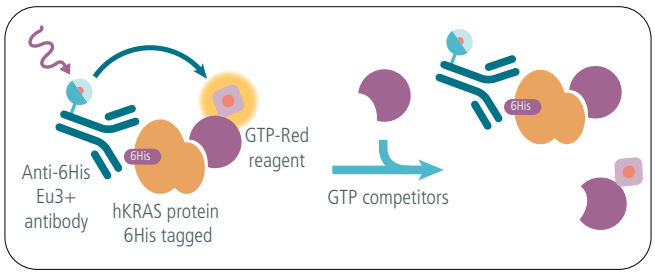


Figure 2: Principle of the KRAS GTP binding assays

Screening of compounds with the KRAS/SOS1 binding kits to identify positive hits and discriminate KRAS G12C/SOS1 binders

A homogeneous and miniaturizable assay protocol to perform the KRAS/SOS1 binding assays

The KRAS/SOS1 binding assays include human tagged recombinant partners (KRAS 4B G12C or WT full length and SOS1 564-1049). In the presence of GTP, the interaction of KRAS and SOS1 is detected by anti-tag reagents for HTRF detection. Compounds which compete with GTP, or binders of the KRAS/SOS1 complex that cause a loss of interaction between both protein partners, will lead to a decrease of the HTRF signal.

These no-wash assays can be carried out in a 96- or 384-well low volume white plate (Revvity # 66PL96100, 20µL final) by adding first the compounds, then a pre-mixed GTP/KRAS G12C or WT solution and the SOS1 protein, and finally the HTRF reagents. All components are incubated for 2h at room temperature before reading the microplate with an HTRF compatible reader, such as the EnVision® or Victor® Nivo™.

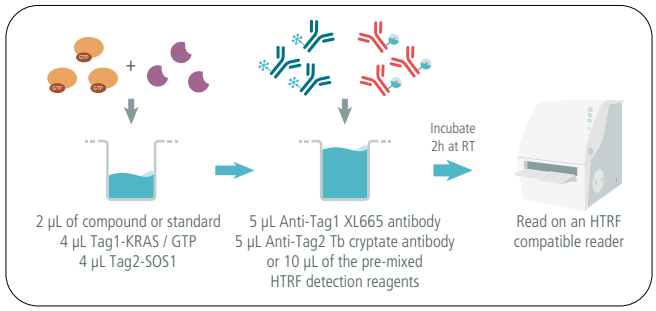


Figure 3: Assay protocol of the HTRF KRAS G12C or WT/SOS1 binding kits

Identification of positive hits with the KRAS/SOS1 binding kits

In order to illustrate the applicability of the KRAS G12C or WT/SOS1 binding kits for the identification of positive hits in a compound screen, several assays with both kits were performed with a panel of KRAS/SOS1 reference inhibitors that have been described in the literature.

A wide range of concentrations of the different inhibitors were dispensed into a 384-well low volume white plate. Then the pre-mixed GTP/KRAS G12C or WT solution, the SOS1 protein and the HTRF detection reagents were added, and the microplate was incubated for 2h at room temperature.

Results obtained with the HTRF KRAS and SOS1 binding kits are shown in Figure 4.

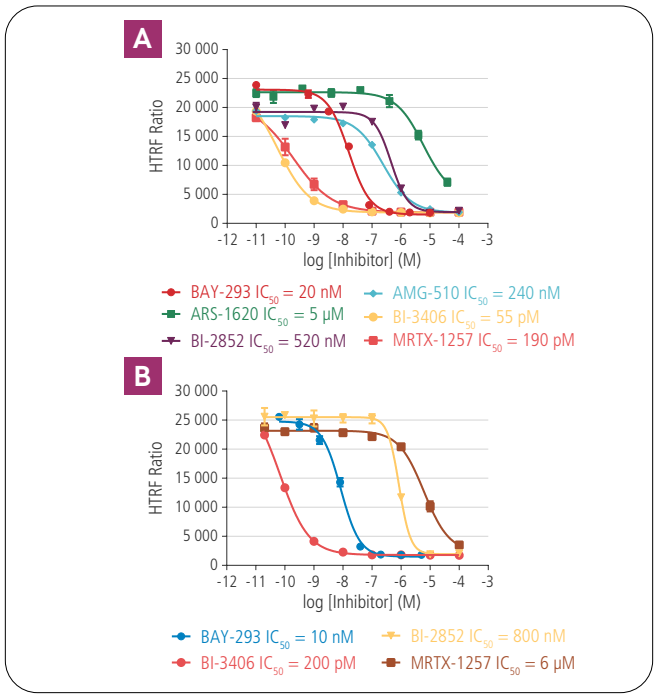


Figure 4: Illustrations with various KRAS/SOS1 reference inhibitors with KRAS G12C/SOS1 (A) and KRAS WT/SOS1 (B) binding kits

As illustrated by the results in Figure 4, the KRAS/SOS1 binding assays enable the identification of positive hits by monitoring the inhibition of KRAS/SOS1 interaction through the loss of HTRF signal due to disruption of KRAS/SOS1 binding.

The comparison of the potency for each inhibitor also proved to be in good correlation with the literature.

The same experiments were also performed with nucleotides, in order to illustrate the applicability of the KRAS/SOS1 binding kits to identify some compounds that are actually GTP competitors as positive hits. In fact, the inhibition of the interaction between KRAS and SOS1 may be disrupted by disturbing the binding of GTP to KRAS (required for the interaction with SOS1).

A wide range of concentrations of GDP (positive control) and ATP (negative control) were dispensed into a 384-well low volume white plate. Then the pre-mixed GTP/KRAS G12C or WT solution, the SOS1 protein, and the HTRF detection reagents were added and the microplate was incubated for 2h at room temperature.

Results obtained with the HTRF KRAS and SOS1 kits are shown in Figure 5.

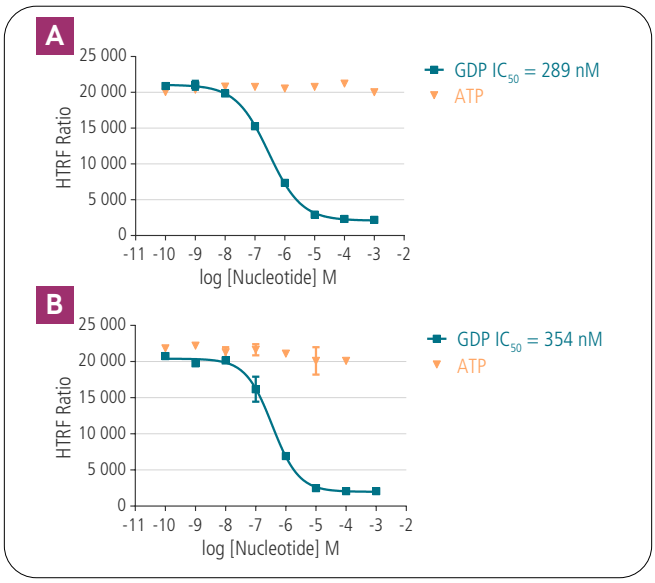


Figure 5: Illustrations with two nucleotides of reference (GDP as positive control; ATP as negative control) and the KRAS G12C/SOS1 (A) and KRAS WT/SOS1 (B) binding kits.

As illustrated in Figure 5, GDP, a KRAS-GTP competitor, shows inhibition of both WT and G12C KRAS/SOS1 binding, demonstrating that GTP competitive compounds will also disrupt the KRAS/SOS1 interaction and can be identified using the HTRF KRAS/SOS1 binding kits.

Discrimination of KRAS G12C/SOS1 binders utilizing the KRAS/SOS1 binding kits

In order to demonstrate the reliability of the KRAS/SOS1 binding kits to discriminate the specific inhibitors of the KRAS G12C/SOS1 complex from the KRAS WT/SOS1 complex, experiments were performed with two inhibitors (ARS-1620 and AMG-510) described in the literature for their specificity in targeting only the KRAS G12C/SOS1 complex. In this context, both inhibitors were tested with the KRAS G12C/SOS1 and the KRAS WT/SOS1 binding kits.

A wide range of concentrations of ARS-1620 and AMG-510 were dispensed into a 384-well low volume white plate. Then the pre-mixed GTP/KRAS G12C or WT solution, the SOS1 protein, and the HTRF detection reagents were added, and the microplate was incubated for 2h at room temperature.

Results obtained with the HTRF KRAS and SOS1 binding kits are shown in Figure 6.

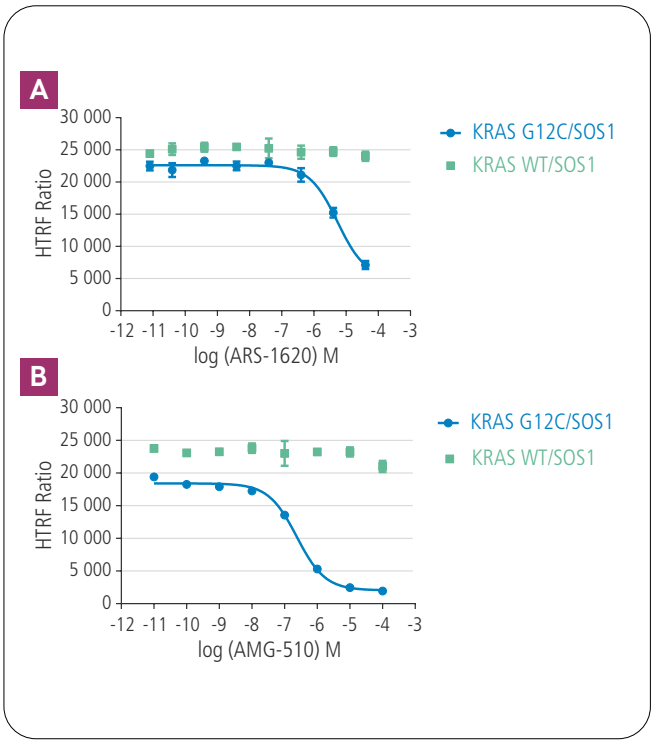


Figure 6: Illustrations with two KRAS G12C/SOS1 selective inhibitors (ARS-1620 (A) and AMG-510 (B)) and the HTRF KRAS/SOS1 binding kits.

As illustrated by the data shown in Figure 6, ARS-1620 and AMG-510 prevent the KRAS/SOS1 interaction with a dose-response effect only for the KRAS G12C/SOS1 complex, proving that using the KRAS/SOS1 binding kits in parallel enables the discrimination of G12C selective compounds.

Taken together, these results demonstrate that the KRAS/ SOS1 binding kits enable the identification of compounds that may be KRAS/SOS1 inhibitors, including both KRAS and SOS1 specific binders, as well as GTP competitors. Moreover, the investigations carried out in parallel with both KRAS/SOS1 binding kits enabled the identification of specific inhibitors to KRAS G12C/SOS1 or KRAS WT/SOS1 complexes.

In order to classify positive hits obtained from screening compounds in the KRAS/SOS1 binding assays, additional investigations can be performed with the HTRF KRAS G12C and WT GTP binding kits in order to identify compounds that are solely GTP competitors.

Counter screening compounds with the KRAS GTP binding kits to specifically identify KRAS GTP competitors

A homogeneous and miniaturizable assay protocol to perform the KRAS GTP binding assays

The KRAS GTP binding assays include human 6His-tagged recombinant KRAS 4B G12C or WT proteins. These assays are in a competitive format which use a GTP-Red reagent labeled with an HTRF acceptor dye as a KRAS ligand, and an anti 6His-Europium-cryptate (HTRF donor) labeled antibody for HTRF detection. In the presence of GTP competitors, the binding of the GTP-Red reagent is prevented, leading to a decrease in the HTRF signal.

These no-wash assays can be carried out in a 96- or 384-well low volume white plate (20µL final), by adding first the compounds, then the KRAS proteins, and finally the HTRF reagents. The mixture is incubated for 1h at room temperature before reading the microplate with an HTRF compatible reader.

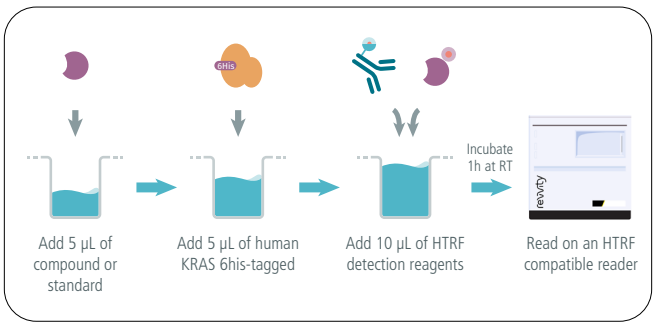


Figure 7: Assay protocol of the HTRF KRAS G12C or WT GTP binding kits

Counter screening of positive hits with the KRAS GTP binding kits

In order to illustrate the applicability of the KRAS G12C or KRAS WT GTP binding kits for the identification of GTP competitors several experiments with both kits were performed with a panel of nucleotides as a reference, and the same compounds as those tested with the KRAS/ SOS1 binding kits.

A wide range of concentrations of the different compounds were dispensed into a 384-well low volume white microplate. Then the KRAS G12C or WT protein and the HTRF detection reagents were added. Finally, the assay was incubated for 1h at room temperature.

Results obtained with the two HTRF KRAS GTP binding kits are shown in Figure 8.

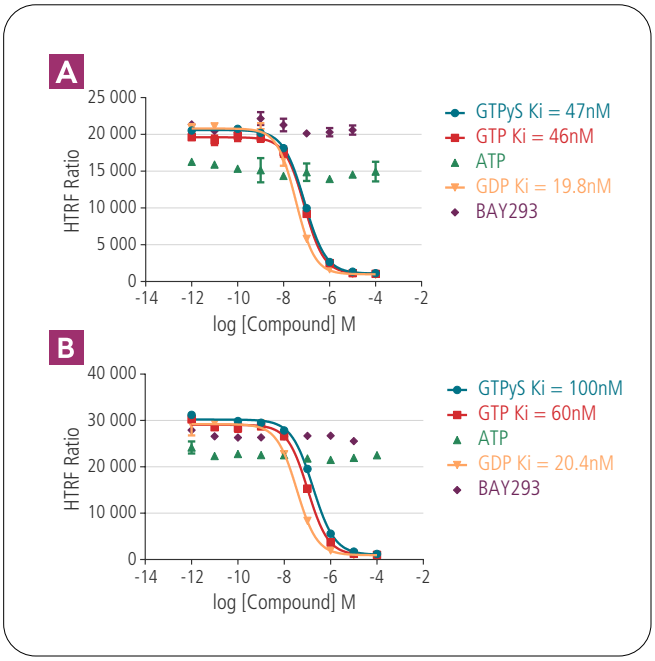


Figure 8: Illustrations with the KRAS G12C (A) and the KRAS WT (B) GTP binding kits with various nucleotides and a KRAS/SOS1 inhibitor of reference.

As illustrated in Figure 8, both HTRF KRAS GTP binding kits enable the discrimination of GTP competitors among a panel of positive hits obtained from the KRAS/SOS1 binding assays and with a consistent pharmacological profile according to the literature.

The guanosine-based nucleotides (GDP, GTPγS, GTP) compete with the GTP-Red reagent, whereas BAY-293, described in the literature as inhibiting the KRAS/SOS1 interaction by binding onto SOS1, does not compete with this reagent.

To further confirm that the HTRF KRAS GTP binding kit is specific for guanosine-based nucleotides, the adenosine-based nucleotide ATP was tested in the assays and no competition was observed.

Taken together, these results demonstrate that the KRAS GTP binding kits are able to identify compounds that are GTP competitors, when used to counterscreen positive hits obtained from the KRAS/SOS1 binding assays.

Conclusion

In this application note we have demonstrated that HTRF biochemical KRAS binding assays enable the rapid identification of KRAS/SOS1 inhibitors in primary compound screens. Using nucleotides and reference compounds widely cited in the literature, we have convincingly shown that the KRAS/SOS1 binding assays discern compounds that disrupt the KRAS/SOS1 interaction. Use of both the KRAS G12C/SOS1 and KRAS WT/SOS1 assays further enables the classification of inhibitors that only target the G12C mutant. Additionally, the KRAS/GTP binding kits can be used to discriminate GTP competitors from inhibitors which may specifically target the KRAS/SOS1 complex. In combination, this new portfolio of HTRF KRAS binding kits makes the characterization of compounds that inhibit KRAS/SOS1 binding possible, and helps further elucidate the mechanism of the biochemical interactions with KRAS, SOS1, and GTP using the robust and powerful HTRF homogeneous assay platform.

References

1. Kessler, D. et al. Druggable and undruggable pockets on KRAS. PNAS. 2019, 32, 15823-15829.
2. Fell, J.B. et al. Identification of the Clinical Development Candidate MRTX849, a Covalent KRASG12C Inhibitor for the Treatment of Cancer. J. Med. Chem. 2020, 63, 6679-6693.
3. Bader, B. et al. Discovery of potent SOS1 inhibitors that block RAS activation via disruption of the RAS-SOS1 interaction. PNAS. 2019, 7, 2551-2560.
4. Janes, M.R. et al. Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. Cell. 2018, 172, 578-589.

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