Modulating KRAS-c-RAF interaction using small molecule inhibitors

Lumit™ Protein Interaction Immunoassay

The Lumit™ Protein Interaction Immunoassay using proteins tags is a homogenous bioluminescent assay to measure protein-protein or protein-small molecule interactions. It combines immunodetection and NanoLuc Binary Technology (NanoBiT®). In Lumit™ Protein Interaction Immunoassays using protein tags, NanoBiT® subunits (SmBiT and LgBiT) are conjugated to two antibodies against common protein tags. When anti-Tag-LgBiT and anti-Tag-SmBiT are added to an interacting protein pair with the corresponding tags, LgBiT and SmBiT are brought into close-proximity to form an active luciferase enzyme that generates luminescence in the presence of substrate (Fig 1A). Lumit™ Protein Interaction Immunoassays have simple, no-wash format (Fig 1B), require small sample volumes, and can be run in a high-throughput format.

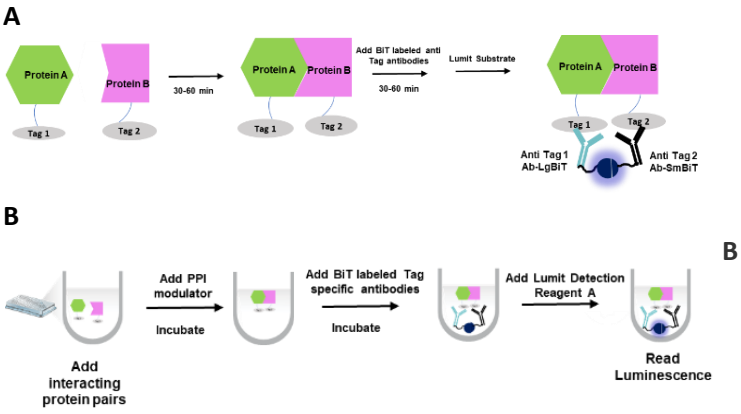

Figure 1. Schematic of Lumit™ Protein Interaction Immunoassay. A. When LgBiT and SmBiT are brought into proximity by the interaction of Protein A and Protein B, an active NanoLuc luciferase generates luminescence in the presence of substrate. **B.** Overview of the simple no-wash protocol.

KRAS as a key therapeutic target

KRAS is a member of the RAS superfamily and regulates cell growth, differentiation, and proliferation. KRAS switches between an inactive and active state through its interactions with GDP and GTP, respectively. These switches are modulated by guanine nucleotide exchange factors (GEFs), which promote GTP binding, and guanine activating proteins (GAPs), which hydrolyze GTP and restart the cycle.

KRAS is one of the most frequently mutated oncogenes in human cancer. The most prevalent mutations occur on codons 12, 13, and 61, and are thought to interfere with GAP-mediated hydrolysis, leading to an accumulation of activated KRAS.

Here, we describe the use of Lumit™ Protein Interaction Immunoassay using protein tags to monitor the interaction of KRAS with effector protein c-RAF (Fig 2).

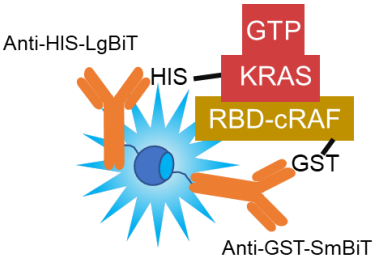

Figure 2. Schematic of KRAS/c-RAF Lumit™ Immunoassay

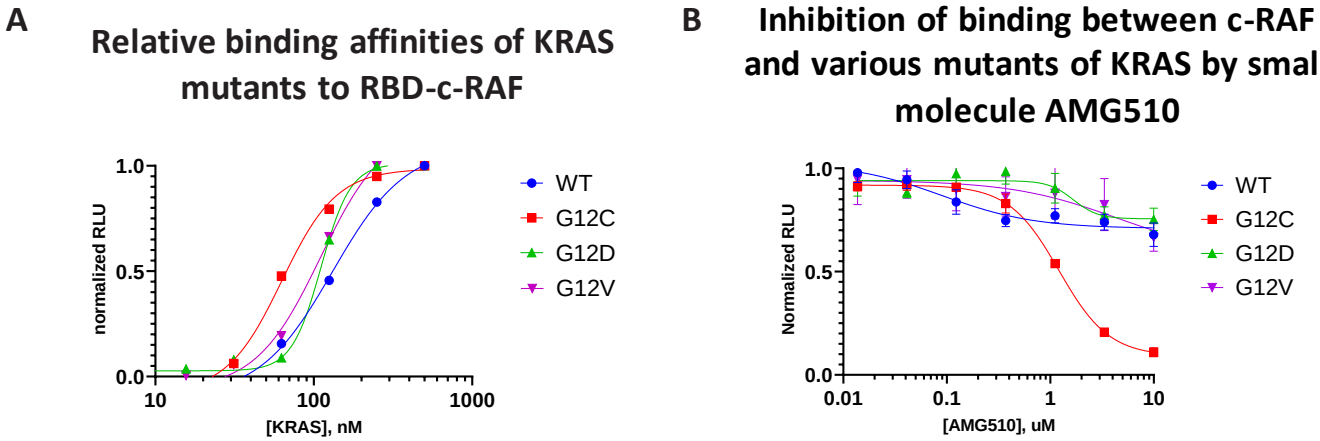

Figure 3. Assessing differences in KRAS mutations for RBD-c-RAF affinity and inhibition with AMG510. *(A) RBD-c-RAF-GST interaction with increasing concentration of KRAS-6HIS (WT or mutant) results in an increase in luminescence signal when probed using BiT-labeled antibodies; (B) AMG510 specifically inhibits KRAS(G12C)/RBD-c-RAF interaction while having little effect on wild-type and other KRAS mutants. Concentrations are expressed as final concentration in each well.*

Lumit™ KRAS-c-RAF Binding Immunoassay

1. Prepare KRAS exchange buffer (1xTBS/5mM EDTA/0.5mM $MgCl_2$/1mM DTT) and add GTP to a final concentration of 10µM.
2. Prepare a dilution series of KRAS and KRAS mutants in KRAS exchange buffer. Add 10µl to each well.
3. Incubate with shaking for 10 minutes to allow formation of activated KRAS-GTP.
4. Add $MgCl_2$ to 10mM to chelate EDTA and stabilize the GTP-bound KRAS.
5. Add 10µl of 200nM RBD-c-RAF diluted in 1x TBS/1mM DTT and incubate for 30min with shaking.
6. Add 20µl of anti-6HIS-LgBiT and anti-GST-SmBiT each diluted to 1.5µg/ml in 1x Lumit™ Immunoassay Dilution Buffer.
7. Incubate with shaking for 30 minutes.
8. Add 10µl of Lumit™ Detection Substrate A diluted 1:50 in Lumit™ Immunoassay Dilution Buffer A to each well.
9. Incubate with shaking for 2 minutes.
10. Read luminescence.
11. For inhibition assay, 200nM of KRAS was preincubated with AMG510 in 1x TBS/1mM DTT and rest of the protocol was as described above.

Materials Needed

Item	Supplier	Cat. #
KRAS, WT, HIS tagged	BPSBioscience	11308
KRAS(G12C), HIS tagged	BPSBioscience	100413
KRAS(G12D), HIS tagged	BPSBioscience	100623
KRAS(G12V), HIS tagged	BPSBioscience	100480
RBD-c-RAF, GST tagged	BPSBioscience	9145
AMG-510	Selleck Chemicals	S8830

Ordering Information

Item	Cat. #
Lumit™ anti-6HIS-LgBiT and -SmBiT	CS332211
Lumit™ anti-GST-LgBiT and -SmBiT	CS332212
Lumit™ Immunoassay Detection Reagent A	VB2010