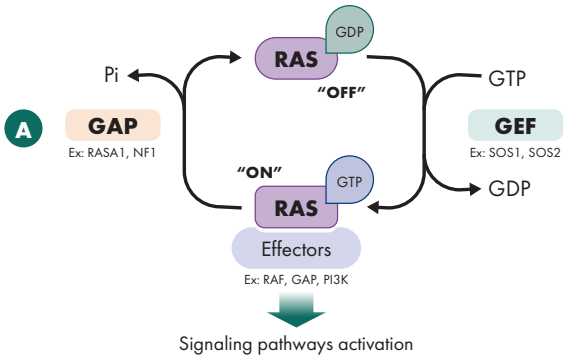
RAS Assays Platform

Comprehensive portfolio of assays and recombinant proteins for the discovery and characterization of compounds that target different steps of the RAS pathway.

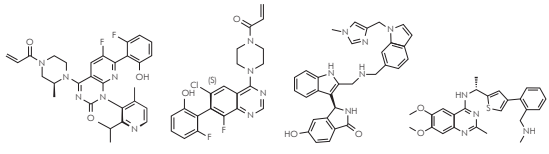
Robust *in vitro* and *in vivo* services for testing of compounds against key RAS targets.

- Nucleotide Exchange Assay
- KRAS Mutant NEA Selectivity Panel
- Protein-Protein Interactions
- TSA and SPR Binding Assays
- Intracellular Target Engagement
- Cellular 3D Assays
- Cell Line Models
- Recombinant Proteins

RAS Activation Pathway



B Main molecules used for assay validation



Brief Overview of Available RAS Assays - Biochemical to *in vivo*

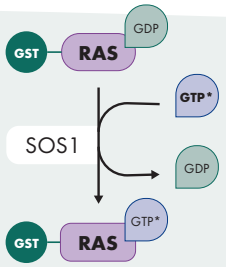
Available Assay	Description
Nucleotide Exchange Assay	The main application of the assay is to identify compounds that lock KRAS in inactive "OFF" state by preventing GTP exchange. Observation of an increase in HTRF signal upon binding of fluorescent GTP to KRAS.
RAS :: cRAF Protein-Protein Interaction (PPI)	HTRF-based assay for testing of compounds that disrupt cRAF binding to RAS.
RAS :: SOS1 Protein-Protein Interaction (PPI)	HTRF-based assay for testing of compounds that disrupt SOS1 binding to RAS.
Surface Plasmon Resonance (SPR) Direct Binding Assay	Determines the kinetics of compounds binding RAS and RAS mutants or SOS.
Thermal Shift Direct Binding Assay	Direct compound binding assay to evaluate compound effect on protein stability.
NanoBRET Target Engagement RAS Assay	Intracellular measurement of the binding affinity of compounds via competitive displacement of a switch I/II pocket tracer.
KRAS Inhibitor Activity Screening – 3D Spheroid Cell Phosphorylation Assay	KRAS inhibitor activity screening using 3D culture and monitoring changes in the activation status of MEK1 by measuring phosphorylation of ERK1/2 (pT202/pY204).
Cell Line Derived Models *in vitro* and *in vivo* models	Drug testing on tumor cells that carry RAS mutations. Reaction Biology provides testing using conventional cell culture as well as cell-line derived xenograft models.

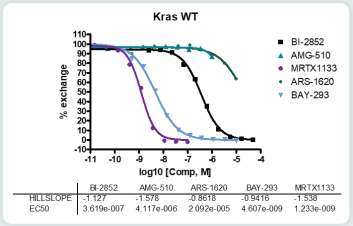
Nucleotide Exchange Assay

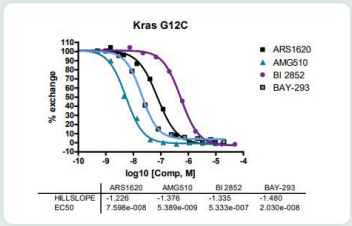
Identify compounds that lock KRAS in inactive "OFF" state by preventing GTP binding.

- Nucleotide exchange assay (NEA) monitors SOS1/2 mediated exchange of GDP to GTP.
- The most frequently used NEA HTRF assay utilizes GTP labelled with DY-647P1 and monitors the increase in HTRF signal observed upon GTP* binding to KRAS.
- The assay is well suited for evaluation of various modes of nucleotide exchange inhibition.
- Also available is the KRAS mutant NEA selectivity panel, designed for screening and profiling inhibitors against KRAS mutants.

HTRF Detection







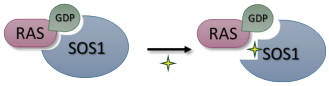
Inhibition of KRAS WT or mutant nucleotide exchange by various control compounds.

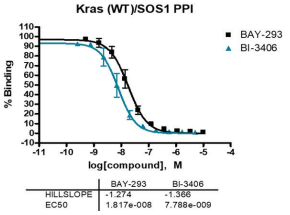
Protein-Protein Interactions

Two assays available to study inhibitors effect on binding of KRAS to SOS1 or cRAF.

RAS::SOS1

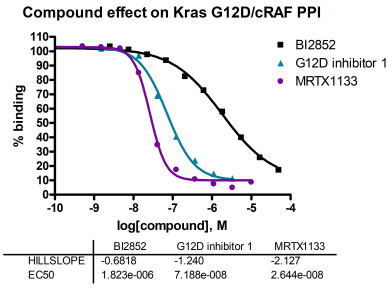
Disruption of SOS1 binding to KRAS can be used as an orthogonal method for studying SOS1 specific compounds. Assay uses HTRF based detection of interaction.





RAS::cRAF

cRAF recognizes the GTP bound form of KRAS. cRAF binding assay can be used for the identification of disruptors of interaction between KRAS and cRAF.



RAS Binding Measurements

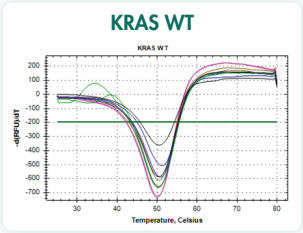
Extensive experience performing TSA and SPR work for a variety of clients.

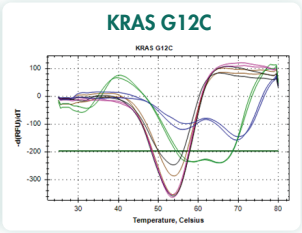
Thermal Shift Assay (TSA)

Assess the effect of compounds on protein stability.

ΔTm	KRAS wt	KRAS G12C Tm1	KRAS G12C Tm2	KRAS G12D	KRAS G12V	SOS1	SOS2
25 uM ARS-1620	0	4.75	11.75	0.5	0.25	0	-0.25
25 uM AMG-510	0.5	3	16.25	0.5	0.25	0	0

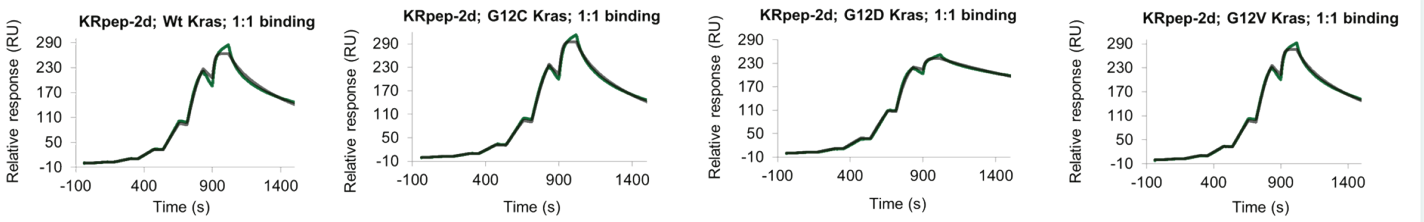
Selectivity of G12C compounds (ARS-1620 and AMG-510) is clearly shown between KRAS wt and mutants.





Surface Plasmon Resonance (SPR)

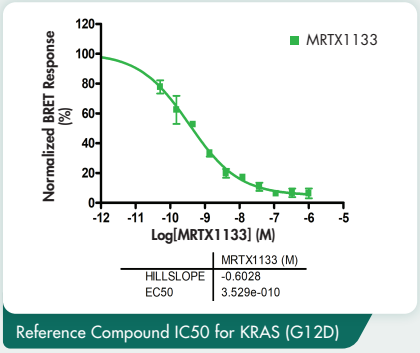
Quantify binding affinity as well as binding kinetics. Comparison between WT and mutant proteins can be performed to determine selectivity. SPR analysis is performed using Biacore 8K instruments. Reaction Biology is a Specialized Center for SPR work in the Chemical Biology Consortium (CBC), of the National Cancer Institute (NCI) Experimental Therapeutics (NExT) Program.



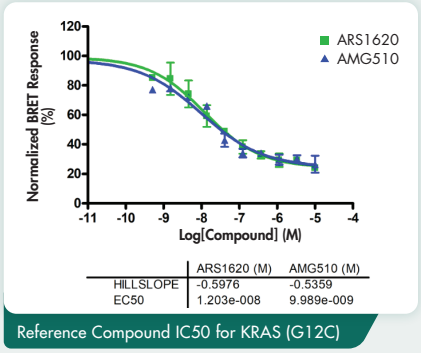
Intracellular RAS Binding via NanoBRET Target Engagement Assay

- Sensitive and efficient method to quantify small molecule drug candidates' binding affinity to the RAS proteins in live cells.
- Some of the key cellular RAS targets established using NanoBRET assay are KRAS, KRAS (G12C), KRAS (G12D), KRAS (G12V), and HRAS.

IC50 data showing the binding potency of clinical inhibitors against KRAS (G12D) and KRAS (G12C).



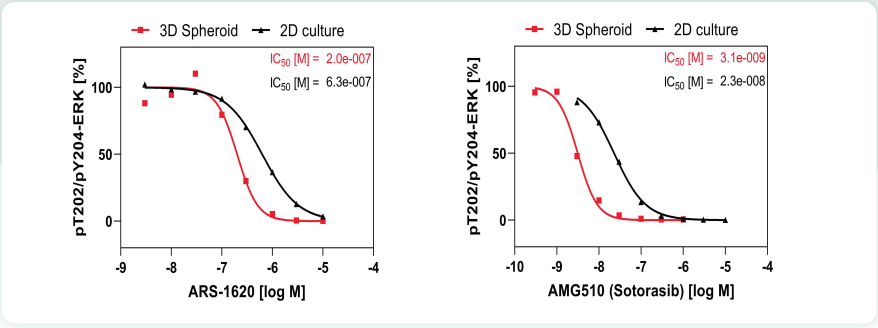
Reference Compound IC50 for KRAS (G12D)



Reference Compound IC50 for KRAS (G12C)

3D Spheroid Cellular Phosphorylation Assay

- Study of the direct effects of KRAS inhibitors against different KRAS mutants in a 3D cell environment. Phosphorylation of ERK1/2 (pT202/pY204) is used as the readout for KRAS activity.
- We established 3D spheroid assays for the analysis of phenotypic effects of KRAS inhibitors and observed that overall KRAS inhibitors demonstrate a higher potency against cells grown in 3D vs 2D.



Available Target Cell Lines

Cell Line	KRAS Mutation
PANC-1	G12D
ASPC-1	G12D
Mia-Pa-Ca-2	G12C
NCI-H358	G12C
SW-620	G12V
BxPC-3	WT
HT-29	WT

Fig. Dose-response curves of MIA-PaCa-2 cells in 3D or 2D culture setup treated with the indicated inhibitors. Cells were cultured and treated for 1, 5 hours (2D) or 3 hours (3D) with indicated inhibitors. Cellular phosphorylation assay was then performed on cell lysates.

Cell Line Derived *in vitro* and *in vivo* models

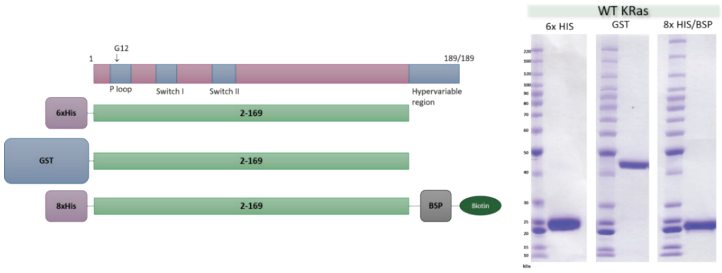
- Perform in vitro or in vivo studies using tumor cells that carry RAS mutations.
- Reaction Biology provides testing on conventional secondary cell culture as well as cell-line derived xenograft models for in vivo pharmacology studies.
- Our cell line-derived xenograft models can be performed with a variety of tumor placement options including subcutaneous, orthotopic, and metastasis models.

Representative example of some of the cell lines available

Tumor Cell Line	KRAS Mutation	HRAS Mutation	NRAS Mutation	Cancer Type	Available as cell model	Available as *in vivo* model
A-498	KRAS G12V	-	-	Kidney cancer	Yes	No
A-549	KRAS G12S	-	-	Lung cancer	Yes	Yes
AN3-CA	-	HRAS F82L	-	Endometrial cancer	Yes	No
AsPC-1	KRAS G12D	-	-	Pancreatic cancer	Yes	Yes
CAL-27	-	-	NRAS D92N, R68T	Oral cancer	Yes	No
Calu-6	KRAS Q61K	-	-	Lung cancer	Yes	Yes

RAS Recombinant Proteins

- KRAS, NRAS, and HRAS recombinant proteins are available with a variety of tags.
- Available for purchase or use in custom services.
- Custom protein production available – please inquire.



Protein Name

BRAF-[RBD] (GST)
CRAF (GST)
CRAF-[RBD] (GST)
HRAS (G12V) BIOTIN
HRAS (G12V) GST
HRAS (G12V) HIS
HRAS (WT) BIOTIN
HRAS (WT) GST
HRAS (WT) HIS
KRAS (G12A) BIOTIN
KRAS (G12A) GST
KRAS (G12A) HIS
KRAS (G12C) BIOTIN
KRAS (G12C) GST
KRAS (G12C) HIS
KRAS (G12D) BIOTIN
KRAS (G12D) GST
KRAS (G12D) HIS
KRAS (G12D-T35S) BIOTIN
KRAS (G12R) BIOTIN
KRAS (G12R) GST
KRAS (G12R) HIS
KRAS (G12S) BIOTIN
KRAS (G12S) GST
KRAS (G12S) HIS
KRAS (G12V) BIOTIN
KRAS (G12V) GST
KRAS (G12V) HIS
KRAS (G13C) BIOTIN
KRAS (G13C) GST
KRAS (G13C) HIS
KRAS (G13D) BIOTIN
KRAS (G13D) GST
KRAS (G13D) HIS
KRAS (Q61H) BIOTIN
KRAS (Q61H) HIS
KRAS (WT) BIOTIN
KRAS (WT) GST
KRAS (WT) HIS
NRAS (Q61H) BIOTIN
NRAS (Q61H) GST
NRAS (Q61H) HIS
NRAS (Q61R) BIOTIN
NRAS (Q61R) GST
NRAS (WT) BIOTIN
NRAS (WT) GST
NRAS (WT) HIS
PI3K-[RBD] (GST)
SOS1
SOS2

List of Available RAS Assays and Proteins

Target	Recombinant Protein	Biochemical assays	Biophysical Assays	Cell-based Assays
HRAS	Yes	NEA; Raf1 PPI	SPR	NanoBRET
HRAS (G12C)	No	Inquire		NanoBRET
HRAS (G12V)	Yes	NEA; Raf1 PPI Assay		NanoBRET
KRAS	Yes	NEA; SOS1 PPI Assay; Raf1 PPI Assay	TSA; SPR	NanoBRET; 3D spheroid
KRAS (G12A)	Yes	NEA and and Raf1 PPI	Inquire	NanoBRET
KRAS (G12C)	Yes	NEA; SOS1 PPI Assay; Raf1 PPI Assay	TSA; SPR	NanoBRET; 3D spheroid
KRAS (G12D)	Yes	NEA; SOS1 PPI Assay; Raf1 PPI Assay	TSA; SPR	NanoBRET; 3D spheroid
KRAS (G12D/T35S)	Yes	NEA		Inquire
KRAS (G12R)	Yes	NEA; Raf1 PPI Assay	SPR	Inquire
KRAS (G12S)	Yes	NEA; Raf1 PPI Assay		NanoBRET
KRAS (G12V)	Yes	NEA; SOS1 PPI Assay; Raf1 PPI Assay	TSA; SPR	NanoBRET; 3D spheroid
KRAS (G13C)	Yes	Inquire		NanoBRET
KRAS (G13D)	Yes	Inquire		NanoBRET
KRAS (Q61H)	Yes	NEA; Raf1 PPI		Inquire
NRAS	Yes	NEA; Raf1 PPI	Inquire	Inquire
NRAS (Q61H)	Yes	NEA; Raf1 PPI		
NRAS (Q61R)	Yes	NEA; Raf1 PPI		

Contact us to inquire about any of these assays We also perform custom assay development.

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