TARGET SPECIFIC ASSAY SERVICES

➢ The Nucleotide Exchange Assay

The nucleotide exchange assay (NEA) monitors SOS1/2 mediated exchange of GDP to GTP. The main application of the assay is to identify compounds that lock K-Ras in inactive "OFF" state by preventing GTP exchange.

The assay is available with two formats:

- Observation of a decrease in fluorescence of labelled GDP (bodypi or Mant) molecules upon it's displacement from K-Ras
- Observation of an increase in HTRF (Homogeneous Time Resolved Fluorescence) upon binding of fluorescent GTP (DY-647P1) to K-Ras

➢ Assay format: GTP binding to K-Ras thereby replacing fluorescent GDP

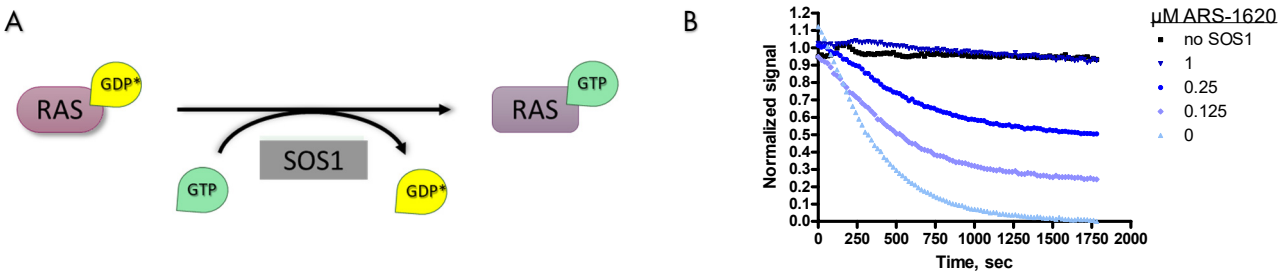
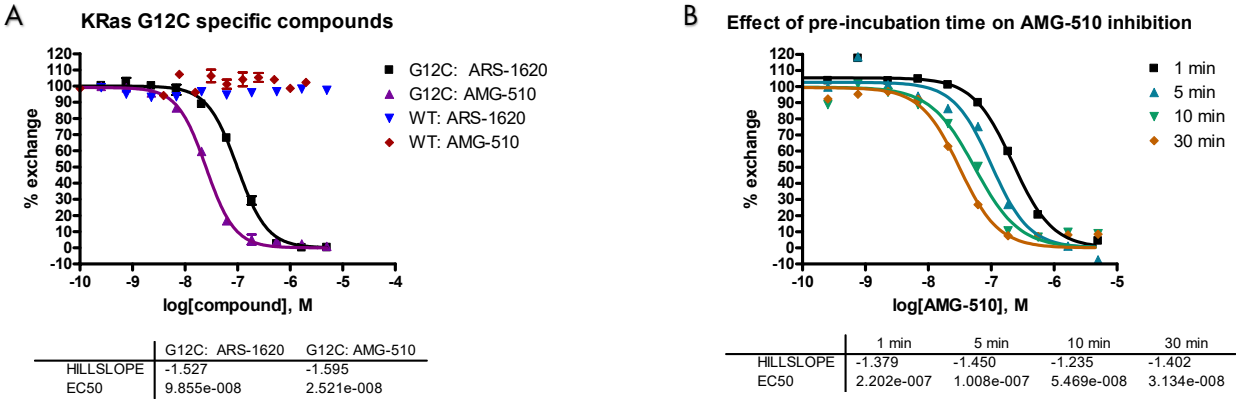


Figure 1. A, Schematic of the nucleotide reaction of fluorescently labelled GDP bound to K-Ras and exchange of GDP* with GTP catalyzed by SOS1. B, decrease in bodipy-GDP fluorescence is observed upon its dissociation from K-Ras. Reference inhibitor ARS-1620 reduces the exchange rate of GDP* to GTP on K-Ras G12C mutant.*



	G12C: ARS-1620	G12C: AMG-510
HILLSLOPE	-1.527	-1.595
EC50	9.855e-008	2.521e-008

	1 min	5 min	10 min	30 min
HILLSLOPE	-1.379	-1.450	-1.235	-1.402
EC50	2.202e-007	1.008e-007	5.469e-008	3.134e-008

Figure 2. A, Example of GDP-replacement assay for selectivity profiling of K-Ras wild type and mutant constructs. B, Measuring the effects of preincubtion time in a time-dependence analysis of compound inhibition of covalent K-Ras G12C specific inhibitor AMG-510.

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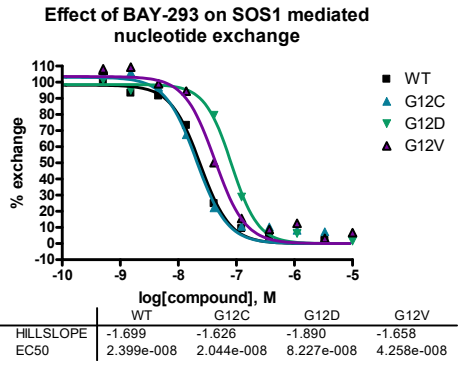
A



	WT	G12C	G12D	G12V
HILLSLOPE	-1.699	-1.626	-1.890	-1.658
EC50	2.399e-008	2.044e-008	8.227e-008	4.258e-008

B

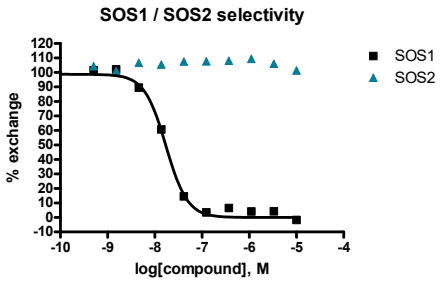


Figure 3. A, The Nucelotide Exchange Assay is well suited for characterization of SOS1 specific compounds at reduced SOS1 concentrations. The difference in BAY-293 IC50 value among different mutants is due to variable required concentration of SOS1 in assay. B, The assay can be utilized for SOS1/2 selectivity testing.

➢ Assay format: HTRF-based nucleotide exchange assay detecting GTP binding to K-Ras

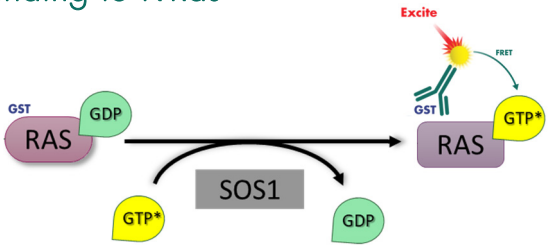
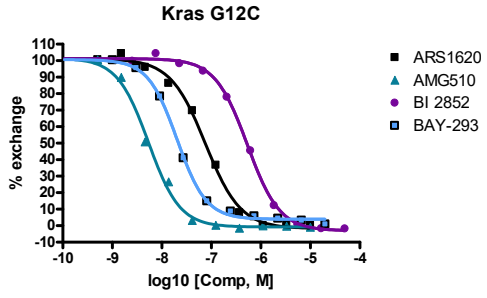


Figure 4. An alternative nucleotide exchange assay detects binding of GTP to K-Ras. During the binding event, DY-647P1-labelled GTP comes in close proximity to GST-tagged K-Ras which is bound to α-GST Tb-cryptate antibody enabling FRET.*

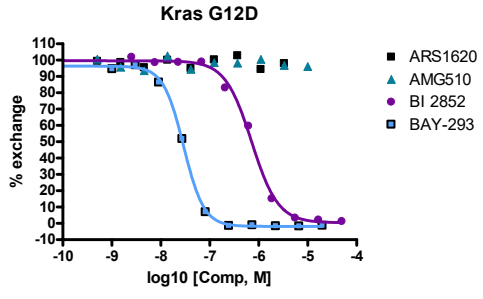
- The HTRF based assay can be used if compounds have fluorescence interference with bodiby-GDP
- Must be performed at lower GTP concentrations
- Can evaluate various modes of nucleotide exchange inhibition

A



	ARS1620	AMG510	BI 2852	BAY-293
HILLSLOPE	-1.226	-1.376	-1.335	-1.480
EC50	7.598e-008	5.389e-009	5.333e-007	2.030e-008

B



	BI 2852	BAY-293
HILLSLOPE	-1.610	-2.098
EC50	7.168e-007	2.930e-008

Figure 5. The HTRF-based nucleotide exchange assay was used for EC50 determination of a variety of reference compounds on KRas mutants G12C (A) and G12D (B).