

## ➤ 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 (bodipy or Mant) molecules upon its 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

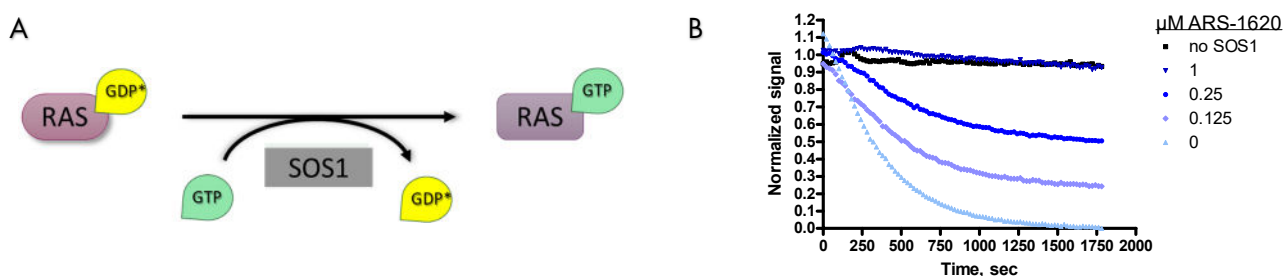


Figure 1. A, Schematic of the nucleotide reaction of fluorescently labelled GDP\* bound to K-Ras and exchange of GDP\* with GTP catalyzed by SOS1. B, decrease in bodipy-GDP fluorescence is observed upon its dissociation from K-Ras. Reference inhibitor ARS-1620 reduces the exchange rate of GDP\* to GTP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

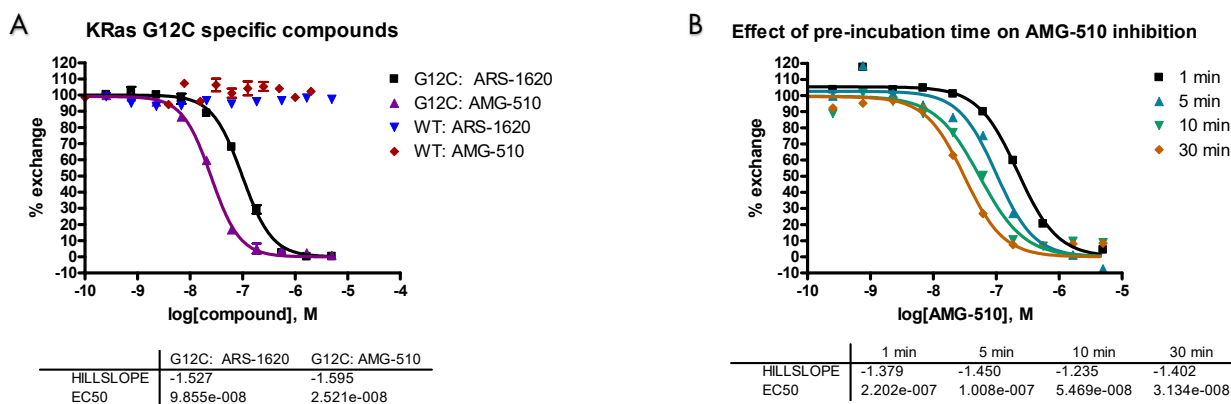


Figure 2. A, Example of GDP-replacement assay for selectivity profiling of K-Ras wild type and mutant constructs. B, Measuring the effects of preincubation time in a time-dependence analysis of compound inhibition of covalent K-Ras G12C specific inhibitor AMG-510.

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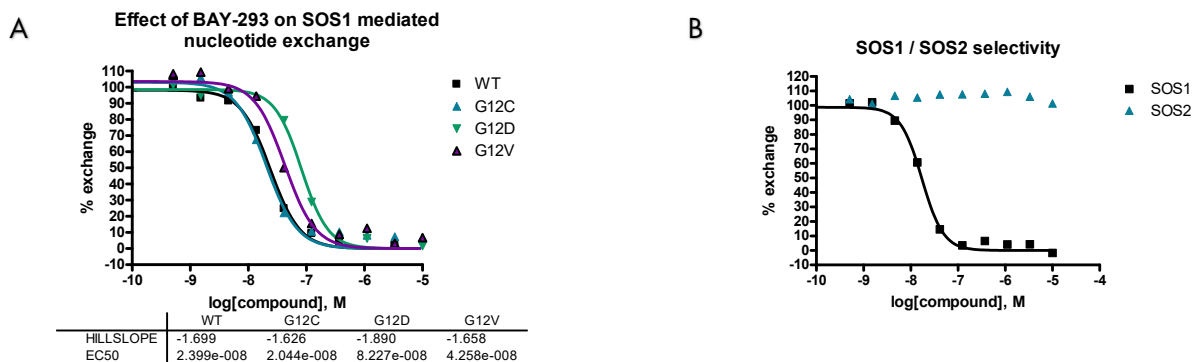


Figure 3. A, The Nucleotide Exchange Assay is well suited for characterization of SOS1 specific compounds at reduced SOS1 concentrations. The difference in BAY-293 IC50 value among different mutants is due to variable required concentration of SOS1 in assay. B, The assay can be utilized for SOS1/2 selectivity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

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

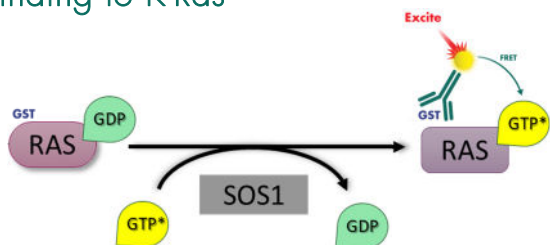


Figure 4. An alternative nucleotide exchange assay detects binding of GTP\* to K-Ras. During the binding event, DY-647P1-labelled GTP comes in close proximity to GST-tagged K-Ras which is bound to  $\alpha$ -GST Tb-cryptate antibody enabling FRET.

- 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

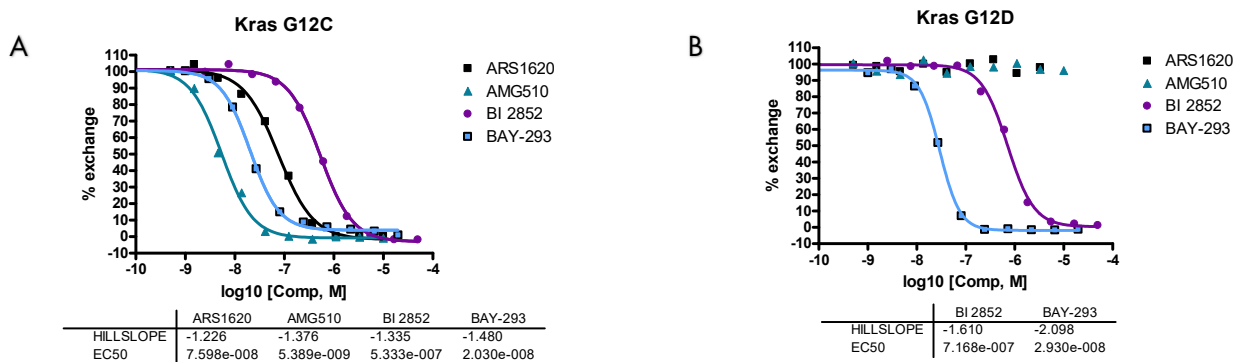


Figure 5. The HTRF-based nucleotide exchange assay was used for EC50 determination of a variety of reference compounds on KRas mutants G12C (A) and G12D (B).