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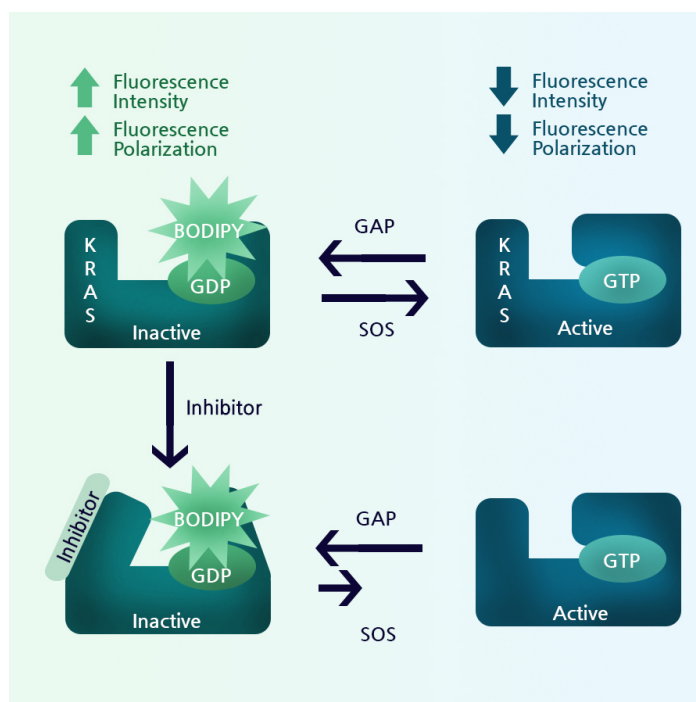
## Data Sheet

### ***KRAS(G12C) Nucleotide Exchange Assay Kit***

**Catalog #79859**  
**384 Reactions**

**Background:** It is well established that RAS mutations are responsible more than 30% of human cancers and KRAS(G12C) is one of the KRAS mutations that is found frequently in lung and colon cancers. Recent studies discovered a small molecule called AMG510 (Amgen) that can block the KRAS(G12C)-mediated signaling pathway by locking the KRAS conformation in the GDP-bound state. Compounds that affect the nucleotide exchange (GDP to GTP) reaction in KRAS could lead to a novel approach to the inhibition of tumor cell growth in KRAS(G12C)-driven tumors

**DESCRIPTION:** The *KRAS(G12C) Nucleotide Exchange Assay Kit* is designed for screening and profiling of KRAS(G12C) antagonists/inhibitors by using BODIPY-GDP to monitor the GDP or GTP binding status. The *KRAS(G12C) Nucleotide Exchange Assay Kit* comes in a convenient 384-well format, with enough purified recombinant *KRAS(G12C)* labeled with BODIPY-GDP, GTP, and assay buffer and additives for 400 enzyme reactions. The kit can be used with two different protocols for greater flexibility, either titrating the inhibitor with fixed GTP concentration or titrating the GTP with fixed inhibitor concentration.



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## COMPONENTS:

Catalog #	Reagent	Amount	Storage	
100537	BODIPY-GDP-loaded KRAS(G12C), 5 $\mu$ M	60 $\mu$ g X 4	-80°C	<b>Avoid multiple freeze/thaw cycles!</b>
79861	GTP (10 mM)	100 $\mu$ l	-20°C	
79862	2X KRAS(G12C) buffer	5 ml	-20°C	
	DTT (0.5 M)	100 $\mu$ l	-20°C	
	EDTA (0.5 M)	100 $\mu$ l	Room Temp	
	384-plate, black	1	Room Temp	

## MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Microplate reader capable of reading Fluorescence (or Fluorescence Polarization)  
Adjustable micropipettor and sterile tips

**APPLICATIONS:** Useful for screening small molecular inhibitors/antagonists that are expected to affect the KRAS(G12C)-GDP or -GTP binding status for drug discovery and HTS targeting of KRAS(G12C).

**STABILITY:** Up to 6 months when stored as recommended.

## REFERENCES:

1. Ostrem, J. M., *et al.*, *Nature*. 2013; **503**:548-551.
2. Patricelli, M. P., *et al.*, *Cancer Discovery*. 2016; **6**:316-329.

## ASSAY PROTOCOL:

*All samples and controls should be tested in duplicate.*

### A. GTP titration at a fixed inhibitor/antagonist concentration

- 1) Thaw **2x KRAS(G12C) buffer**, **DTT (0.5 M)** and **BODIPY-GDP loaded KRAS(G12C)**.

*(Note: **BODIPY-GDP loaded KRAS(G12C)** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. We do not recommend re-using thawed aliquots)*

- 2) Prepare 1X KRAS(G12C) buffer containing 1 mM DTT (e.g. for 4 ml 1X KRAS buffer, mix 2 ml **2X KRAS(G12C) buffer** + 8  $\mu$ l **DTT (0.5 M)** + 1,992  $\mu$ l H<sub>2</sub>O)
- 3) Prepare the master solution (15  $\mu$ l): N wells X (5  $\mu$ l **BODIPY-GDP loaded KRAS(G12C)** + 10  $\mu$ l 1X KRAS(G12C) buffer prepared above). Add 15  $\mu$ l of the master solution to all wells.

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- 4) Add 5  $\mu$ l of test inhibitor to each of the wells. For control wells, add 5  $\mu$ l of the same buffer without inhibitor (inhibitor buffer). Inhibitor buffer should contain the same concentration of DMSO as the test inhibitor. For example, prepare 100x test compound in DMSO; dilute 1:20 in water to make a 5% DMSO solution. For the control, use 5% DMSO(aq) with no inhibitor.

	<b>Positive Control</b>	<b>Test Inhibitor</b>
1x KRAS(G12C) buffer	10 $\mu$ l	10 $\mu$ l
BODIPY-GDP loaded KRAS(G12C)	5 $\mu$ l	5 $\mu$ l
Test Inhibitor	-	5 $\mu$ l
Inhibitor Buffer (e.g. 5% DMSO (aq))	5 $\mu$ l	-
Incubate for 2 hours at room temperature		
GTP (0 mM-1 mM)	2.5 $\mu$ l	2.5 $\mu$ l
EDTA (25 mM)	2.5 $\mu$ l	2.5 $\mu$ l
<b>Total</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>

*Note: In the GTP titration experiment, a fixed concentration of the inhibitor is used. e.g. for AMG510, 10  $\mu$ M is recommended).*

- 5) Centrifuge the plate to ensure all the components are mixed well and incubate the plate for 2 hours at room temperature.
- 6) Thaw **GTP (10 mM)** and **EDTA (0.5 M)**. Make 3-fold serial dilutions of GTP in H<sub>2</sub>O from 0 mM to 1 mM. (These dilutions are 10X concentration, so the final GTP concentration in the reaction will be 0 to 100  $\mu$ M).
- 7) Dilute **EDTA (0.5 M)** in H<sub>2</sub>O to prepare 25 mM EDTA, e.g. to make 1 ml of 25 mM EDTA (aqueous), mix 50  $\mu$ l **EDTA (0.5M)** with 950  $\mu$ l H<sub>2</sub>O.
- 8) After 2-hour incubation, add 2.5  $\mu$ l of the serially diluted GTP to the wells.
- 9) Initiate the reaction by adding 2.5  $\mu$ l of 25 mM EDTA prepared above (final concentration of EDTA is 2.5 mM). Centrifuge the plate to ensure the all the components are mixed well and incubate the plate for 1 hour at room temperature.
- 10) After 1-hour incubation, read the Fluorescence at Ex<sub>470nm</sub>/Em<sub>525nm</sub>. Alternatively, Fluorescence Polarization can be measured at the same wavelength.

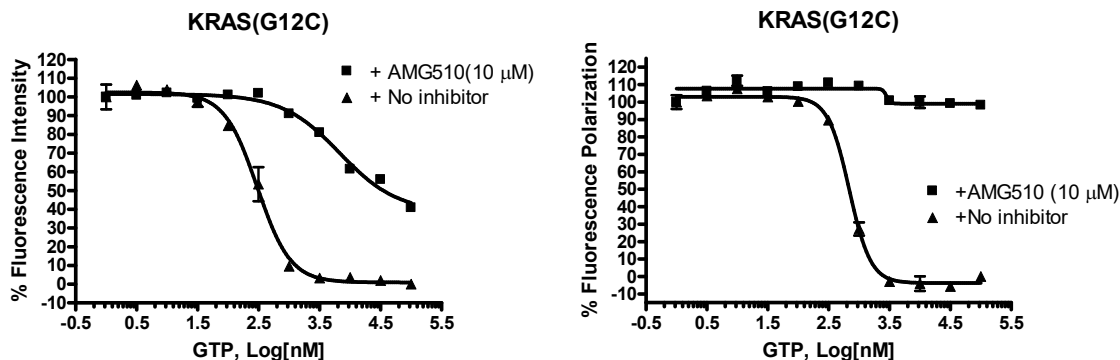
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### Example of Assay Results:



EDTA mediating GDP-GTP exchange reaction of KRAS(G12C). Assay was performed by following the *KRAS(G12C) Nucleotide Exchange Assay Kit* protocol. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

### ***B. Inhibitor titration at a fixed GTP concentration***

- 1) Thaw **2x KRAS(G12C) buffer**, **DTT (0.5 M)** and **BODIPY-GDP loaded KRAS(G12C)**.

*(Note: **BODIPY-GDP loaded KRAS(G12C)** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. We do not recommend re-using thawed aliquots)*

- 2) Prepare 1X KRAS(G12C) buffer containing 1 mM DTT (e.g. for 4 ml 1X KRAS buffer, mix 2 ml **2X KRAS(G12C) buffer** + 8 μl **DTT (0.5 M)** + 1,992 μl H<sub>2</sub>O)
- 3) Prepare the master solution (15 μl): N wells X (5 μl **BODIPY-GDP loaded KRAS(G12C)** + 10 μl 1X KRAS(G12C) buffer prepared above).
- 4) Add 15 μl of the master solution to each well.
- 5) Make 3-fold serial dilutions of the inhibitor in 5% DMSO (aq). This is the 10X intermediate dilution of the inhibitor. Note: These dilutions are 10X concentration. For example, the highest testing concentration of AMG510 would be 10 μM, so prepare the dilutions from 0 μM to 100 μM in 5% DMSO (aq).

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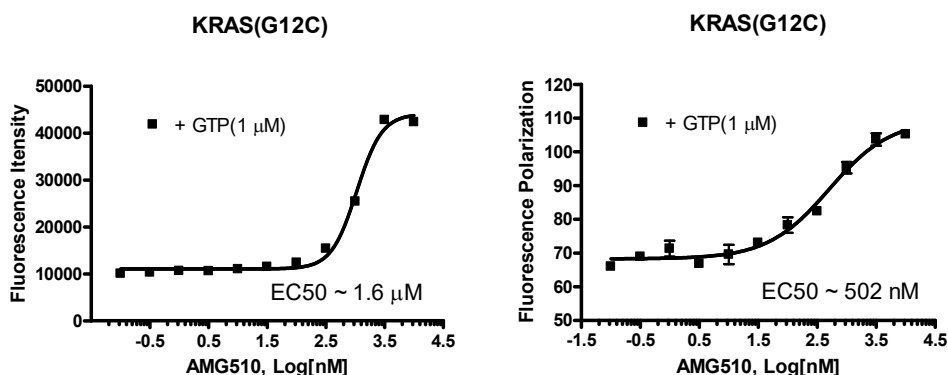
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- 6) Add 5  $\mu$ l of the diluted inhibitor to the wells and centrifuge the plate to ensure all the components are mixed well.
- 7) Incubate the plate for 2 hours at room temperature.
- 8) Thaw **GTP (10 mM)** and **EDTA (0.5 M)**. Prepare 10  $\mu$ M GTP in H<sub>2</sub>O [e.g. Dilute **GTP (10 mM)** 10-fold in H<sub>2</sub>O (10  $\mu$ l **GTP(10 mM)** + 90  $\mu$ l H<sub>2</sub>O) to prepare 1 mM GTP, and dilute it 100 fold more to make a 10  $\mu$ M solution (10  $\mu$ l **GTP(1 mM)** + 990  $\mu$ l H<sub>2</sub>O)].
- 9) Dilute **EDTA (0.5 M)** in H<sub>2</sub>O to prepare 25 mM EDTA, e.g. for 1 ml of 25 mM EDTA, mix 50  $\mu$ l **EDTA (0.5M)** with 950  $\mu$ l H<sub>2</sub>O.
- 10) Mix diluted GTP (10  $\mu$ M) and EDTA (25 mM) at 1:1 ratio.
- 11) After 2-hour incubation, initiate reaction by adding 5  $\mu$ l of the GTP/EDTA solution prepared in **step 10**), and incubate the plate for 1-hour at room temperature.
- 12) After 1-hour incubation, read the Fluorescence at Ex<sub>470nm</sub>/Em<sub>525nm</sub>. Alternatively, Fluorescence Polarization can be measure at the same wavelength.

### Example of Assay Results:



EDTA mediating GDP-GTP exchange reaction of KRAS(G12C). Assay was performed by following the *KRAS(G12C) Nucleotide Exchange Assay Kit* protocol. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

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**RELATED PRODUCTS:**

<b><u>Product Name</u></b>	<b><u>Catalog #</u></b>	<b><u>Size</u></b>
KRAS, His-Tag	11308	100 µg
KRAS (G12V), His-Tag	100480	100 µg
KRAS (G13D), His-Tag	100479	100 µg
KRAS (G12C), His-Tag	100413	100 µg
p38α, GST-tag	40070	10 µg

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