# KRAS-G12C/VHL PROTAC BINDING ASSAY KITS

PROTOCOL

**Part #** 63ADK000CB30PEG & 63ADK000CB30PEH

**Test size:** 500 tests (63ADK000CB30PEG), 10,000 tests (63ADK000CB30PEH) - assay volume: 20 µL

**Revision:** 01 (May 2021)

**Store at:** -60°C or below

**For research use only. Not for use in diagnostic procedures.**

## ASSAY PRINCIPLE

The HTRF KRAS-G12C/VHL PROTAC Binding Assay is designed to measure the ternary complex formation between KRAS-G12C, PROTAC degrader and VHL proteins. Utilizing HTRF (Homogeneous Time-resolved Fluorescence) technology, the assay enables simple and rapid characterization of PROTAC degraders in a high throughput format.

As shown in Figure 1, the interaction between Tag1-KRAS-G12C and Tag2-VHL is detected by using anti-Tag1-Europium (HTRF donor) and anti-Tag2-XL665 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to the KRAS-G12C, PROTAC degrader and VHL binding, excitation of the donor antibody triggers fluorescent resonance energy transfer (FRET) towards the acceptor antibody, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the extent of PROTAC degrader bind to both KRAS-G12C and VHL protein. Thus, PROTAC degrader forming a ternary complex will cause an increase in HTRF signal.

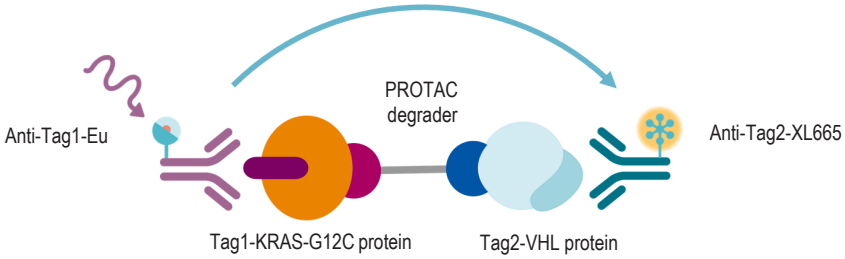
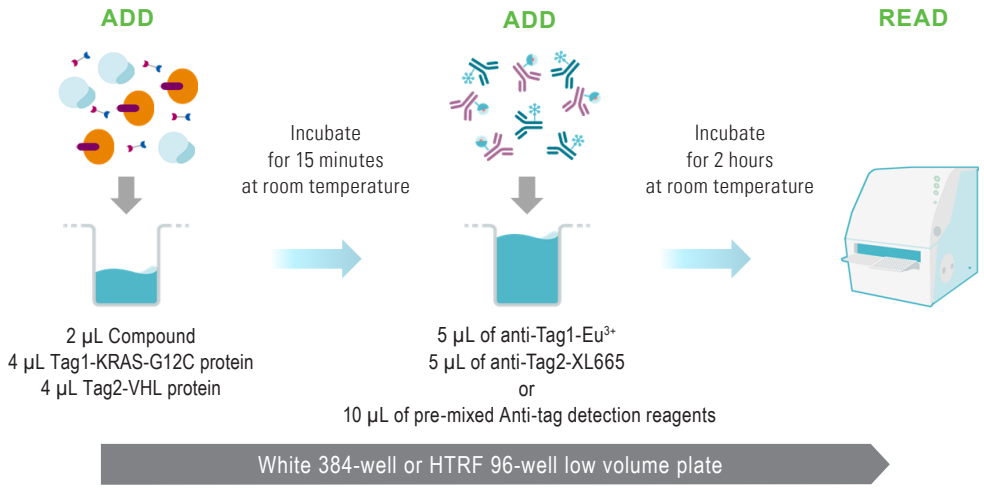



Figure 1: Principle of the HTRF KRAS-G12C/VHL PROTAC binding assay.

## PROTOCOL AT A GLANCE




Make sure to use the setup for $Eu^{3+}$ Cryptate. For more information about set-up and compatible HTRF® readers, please visit our website at: http://www.cisbio.com/readers

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## MATERIALS:

| KIT COMPONENTS | 500 TESTS<br>CAT # 63ADK000CB30PEG | 10,000 TESTS<br>CAT # 63ADK000CB30PEH |
|---|---|---|
| Tag1-KRAS-G12C*<br>MW: 45.5 kDa | 1 vial<br>50 µL 250X<br>Frozen | 1 vial<br>840 µL 250X<br>Frozen |
| Tag2-VHL (CUL2/ELOB/ELOC/RBX1) Complex*<br>MW: 153.0 kDa | 1 vial<br>25 µL 500X<br>Frozen | 1 vial<br>420 µL 500X<br>Frozen |
| PROTAC Standard<br>MW: 1132.8 Da | 1 vial<br>10 µL 3 mM DMSO Solution<br>Frozen | 2 vials<br>10 µL 3 mM DMSO Solution<br>Frozen |
| Anti-Tag1-$Eu^{3+}$ | 1 vial<br>25 µL 100X<br>Frozen | 1 vial<br>0.5 mL 100X<br>Frozen |
| Anti-Tag2-XL665 | 1 vial<br>25 µL 100X<br>Frozen | 1 vial<br>0.5 mL 100X<br>Frozen |
| Diluent | 1 vial 20 mL<br>Cat# 62DLBDDD (200 mL)<br>ready-to-use | 1 vial 200 mL<br>Cat# 62DLBDDD (200 mL)<br>ready-to-use |
| Detection Buffer | 1 vial 10 mL<br>Cat# 62DB1FDG (130 mL)<br>ready-to-use | 1 vial 130 mL<br>Cat# 62DB1FDG (130 mL)<br>ready-to-use |

* The amounts of Tag1-KRAS-G12C and Tag2-VHL provided are sufficient for the validated amounts of tagged proteins suitable for PROTAC ternary complex formation study: optimized concentrations of KRAS-G12C and VHL in 20 µL final assay volume.

For reading, an HTRF®-Certified Reader is needed.
For HTRF microplate recommendations, please visit http://www.cisbio.com/microplate-recommendations
For a list of HTRF-compatible readers and setup recommendations, please visit http://www.cisbio.com/readers

## STORAGE AND STABILITY

Store the kit at -60°C or below. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

Reagents

Once thawed, tagged KRAS-G12C & VHL stock solution may be frozen, and can be thawed only once.
Once thawed (or reconstituted), anti-Tag solutions can be frozen once.
To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.

Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

## REAGENT PREPARATION

### BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature.
- Before use, allow all reagents to warm up to room temperature then homogeneize buffer and diluent. It is recommended to filter buffers before use.
- The tagged protein solutions must be prepared in individual vials - DO NOT premix tagged solutions prior to dispensing.
- The anti-Tag solutions must be prepared in individual vials and can be premix prior to dispensing.
- Compounds may be prepared in diluent. We recommend keeping DMSO below 1% during the assay (20 µL final volume).

## TO PREPARE WORKING SOLUTIONS:

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

| 500 TESTS | 10,000 TESTS |
|---|---|
| Tag1-KRAS-G12C protein<br>250X stock solution of optimized final concentration | |
| Thaw the Tag1-KRAS-G12C protein* solution.<br>Dilute 50-fold the 250X Tag1-KRAS-G12C protein stock solution with diluent buffer to prepare a 5X working solution.<br>e.g. 10 µL of thawed Tag1-KRAS-G12C protein stock solution + 490 µL of diluent buffer. (20 µL final volume). | |
| Tag2-VHL protein<br>500X stock solution of optimized final concentration | |
| Thaw the Tag2-VHL protein* solution.<br>Dilute 100-fold the 500X Tag2-VHL protein stock solution with diluent buffer to prepare a 5X working solution.<br>e.g. 5 µL of thawed Tag2-VHL protein stock solution + 495 µL of diluent buffer. (20 µL final volume). | |
| Anti-Tag1-$Eu^{3+}$ | |
| Thaw the anti-Tag1-$Eu^{3+}$ solution.<br>This 100X stock solution can be frozen and stored at -60°C or below.<br>Dilute 100-fold the 100X anti-Tag1-$Eu^{3+}$ stock solution with detection buffer. | |
| e.g. 25 µL of thawed anti-Tag1-$Eu^{3+}$ stock solution + 2475 µL of detection buffer. | e.g. 0.5 mL of thawed anti-Tag1-$Eu^{3+}$ stock solution + 49.5 mL of detection buffer. |
| Anti-Tag2-XL665 | |
| Thaw the anti-Tag2-XL665 solution.<br>This 100X stock solution can be frozen and stored at -60°C or below.<br><br>Dilute 100-fold the 100X anti-Tag2-XL665 stock solution with detection buffer.<br>e.g. 25 µL of thawed anti-Tag2-XL665 stock solution + 2475 µL of detection buffer. | Thaw the anti-Tag2-XL665 solution.<br>This 100X stock solution can be frozen and stored at -60°C or below.<br><br>Dilute 100-fold the 100X anti-Tag2-XL665 stock solution with detection buffer.<br>e.g. 0.5 mL of reconstituted anti-Tag2-XL665 stock solution + 49.5 mL of detection buffer. |

*Titration of Tag1-KRAS-G12C or Tag2-VHL can be performed if necessary.

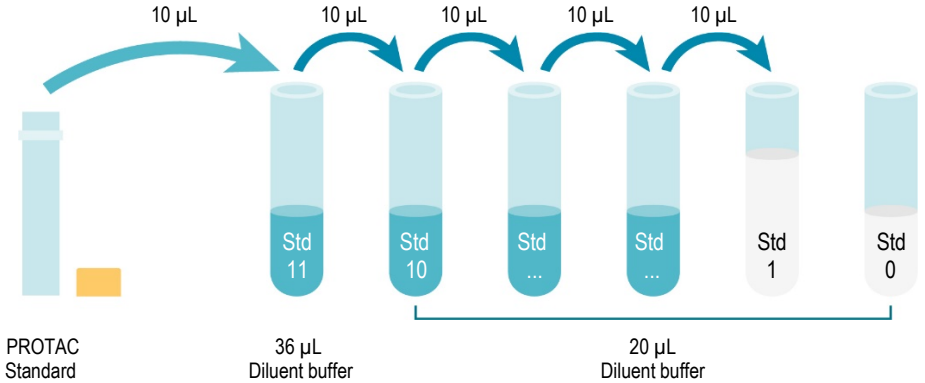
## TO PREPARE WORKING PROTAC STANDARD SOLUTIONS:

- Each well requires 2 µL of standard.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

### A recommended standard dilution procedure is listed and illustrated below:

- Dilute the PROTAC standard stock solution 10-fold with diluent buffer to prepare high standard (Std 11):
  Take 4 µL of standard stock solutionand add it to 36 µL of diluent buffer. Mix gently.
- Use the high standard (Std 11) to prepare the standard curve using 3-fold serial dilutions, as follows:
  - Dispense 20 µL of diluent buffer into each vial from Std 11 to Std 0.
  - Add 10 µL of standard to 20 µL of diluent buffer, mix gently, and repeat the serial dilution to make the other standard solutions: Std10, Std9, Std8, Std7, Std6, Std5, Std4, Std3, Std2, Std1.

This will create 11 standards for the analyte. Std 0 (Negative control) is diluent buffer.



| STANDARD | SERIAL DILUTIONS | WORKING SOLUTIONS | FINAL CONCENTRATIONS |
|---|---|---|---|
| Standard stock solution | Thawed stock solution | 3 000 µM | |
| Standard 11 | 4 µL standard stock solution + 36 µL Diluent buffer | 300 µM | 30 µM |
| Standard 10 | 10 µL Standard 11 + 20 µL Diluent buffer | 100 µM | 10 µM |
| Standard 9 | 10 µL Standard 10 + 20 µL Diluent buffer | 33 µM | 3.3 µM |
| Standard 8 | 10 µL Standard 9 + 20 µL Diluent buffer | 11 µM | 1.1 µM |
| Standard 7 | 10 µL Standard 8 + 20 µL Diluent buffer | 3.7 µM | 0.37 µM |
| Standard 6 | 10 µL Standard 7 + 20 µL Diluent buffer | 1.2 µM | 0.12 µM |
| Standard 5 | 10 µL Standard 6 + 20 µL Diluent buffer | 0.41 µM | 0.041 µM |
| Standard 4 | 10 µL Standard 5 + 20 µL Diluent buffer | 0.14 µM | 0.014 µM |
| Standard 3 | 10 µL Standard 4+ 20 µL Diluent buffer | 0.046 µM | 0.0046 µM |
| Standard 2 | 10 µL Standard 3 + 20 µL Diluent buffer | 0.015 µM | 0.0015 µM |
| Standard 1 | 10 µL Standard 2 + 20 µL Diluent buffer | 0.005 µM | 0.0005 µM |
| Standard 0 | 20 µL Diluent buffer | 0 µM | 0 µM |

## ASSAY PROTOCOL

| | Cryptate control | Standard (Std 0 - Std 11) | Compound |
|---|---|---|---|
| Step 1 | Dispense 10 µL of diluent buffer into each cryptate control well. | Dispense 2 µL of each PROTAC standard (Std 0 - Std 11) into each standard well. | Dispense 2 µL of compound into each compound well. |
| Step 2 | | Add 4 µL of Tag1-KRAS-G12C protein and 4 µL of Tag2-VHL protein to all wells | |
| Step 3 | Incubate for 15 minutes at room temperature. | | |
| Step 4 | Dispense 5 µL of anti-Tag1-Eu3+ and 5 µL of detection buffer. | Dispense 10 µL of pre-mixed anti-Tag1-Eu3+ and anti-Tag2-XL665. | |
| Step 5 | Seal the plate and incubate for 2 hours at room temperature. | | |
| Step 6 | Remove the plate sealer and read on an HTRF® compatible reader. | | |

## EXAMPLE OF PLATE MAP

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | **Buffer control**:<br>10 µL diluent<br>10 µL detection buffer | Repeat Well A1 | Repeat Well A1 | **Compound...:**<br>2 µL compound...<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well A4 | Repeat Well A4 |
| B | **Cryptate control:**<br>10 µL diluent<br>5 µL detection buffer<br>5 µL anti-Tag1-Eu | Repeat Well B1 | Repeat Well B1 | **Compound...:**<br>2 µL compound...<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well B4 | Repeat Well B4 |
| C | **Negative control:**<br>2 µL diluent<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well C1 | Repeat Well C1 | **Compound...:**<br>2 µL compound...<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well C4 | Repeat Well C4 |
| D | **Positive control:**<br>2 µL PROTAC standard<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well D1 | Repeat Well D1 | **Compound...:**<br>2 µL compound...<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well D4 | Repeat Well D4 |
| E | **Compound 1:**<br>2 µL compound 1<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well E1 | Repeat Well E1 | **Compound...:** | Repeat Well E4 | Repeat Well E4 |
| F | **Compound 2:**<br>2 µL compound 2<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well F1 | Repeat Well F1 | **Compound...:** | Repeat Well F4 | Repeat Well F4 |
| G | **Compound...:**<br>2 µL compound...<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well G1 | Repeat Well G1 | **Compound...:** | Repeat Well G4 | Repeat Well G4 |
| H | **Compound...:**<br>2 µL compound...<br>4 µL Tag1-KRAS-G12C<br>4 µL Tag2-VHL<br>10 µL pre-mix anti-Tag reagents | Repeat Well H1 | Repeat Well H1 | | | |

## DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit http://www.cisbio.com/data-reduction

**The data should be fitted with Bell-shaped equation in GraphPad Prism software.**

## RESULTS

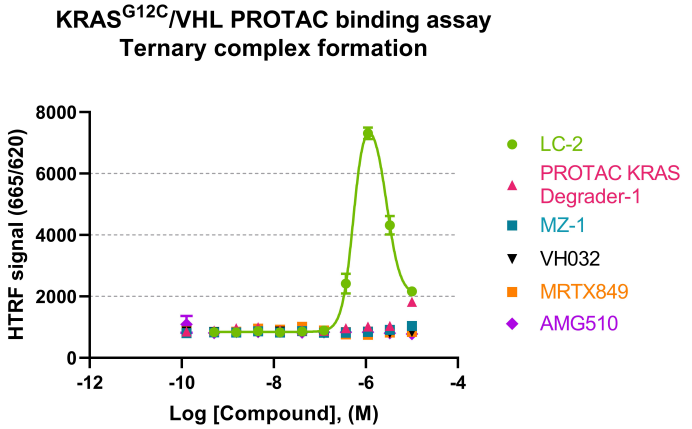
The data shown below must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

The formation of ternary complex were tested at optimized concentrations of VHL and KRAS-G12C.

Readouts on **PerkinElmer® EnVision** with a flash lamp.

Note that results may vary from one HTRF® compatible reader to another.

| Compound | Vender | Cat# | Description |
|---|---|---|---|
| LC-2 | MCE | HY-137516 | A potent and first-in-class PROTAC which can degrade endogenous KRAS-G12C and with a MRTX849 warhead. |
| K-Ras Degrader-1 | MCE | HY-129523 | A potent K-Ras degrader based PROTAC between KRAS G12C and CRBN. |
| MZ-1 | MCE | HY-107425 | A long-lasting and selective removal degrader based PROTAC between BRD4 and VHL. |
| VH032 | MCE | HY-120217 | A ligand used in the recruitment of the VHL protein. It is used as a precursor to synthesize LC-2. |
| MRTX849 | MCE | HY-130149 | A potent and orally-available covalent inhibitor of KRAS G12C with potential antineoplastic activity. |
| AMG510 | Selleck | S8830 | An effective covalent inhibitor of KRAS KRAS G12C which has potential anti-tumor activity. |




The concentration of LC-2 at peak, 1111 nM



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