NanoBRET™ TE Intracellular Kinase Assay

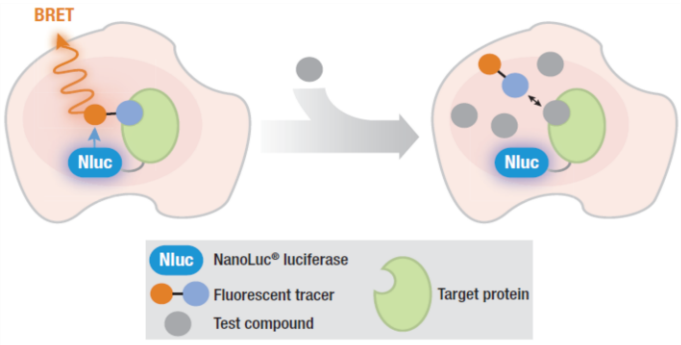
PIK3CA(H1047Y)/PIK3R1 TE Assay

Assay Format: ADH (1)
NanoBRET™ Tracer: K-3 (2)
100X [Tracer]: 3.1µM in DMSO
Final [Tracer]: 0.031µM
Assay Category: High Window (3)
Z': 0.78

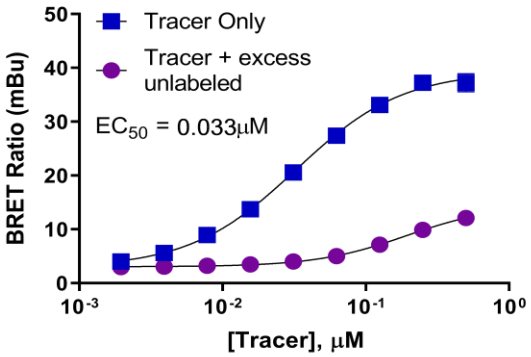
Materials Needed

- **NanoLuc®-PIK3CA(H1047Y) Fusion Vector (Cat.# NV3971)**
- **PIK3R1 Expression Vector (Cat.# NV4031)**
- **NanoBRET™ TE Intracellular Kinase Assay, K-3 (Cat.# N2600, N2601, N2810; 2)**

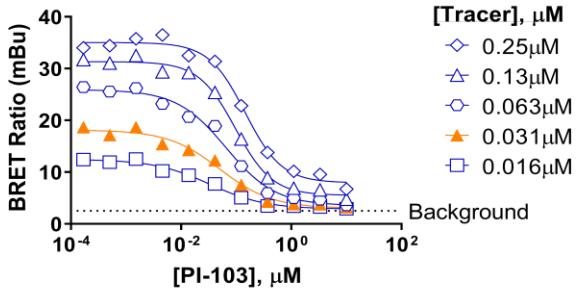
Overview of the NanoBRET TE Assay



NanoLuc-PIK3CA(H1047Y)/PIK3R1 Tracer K-3 Dose Response

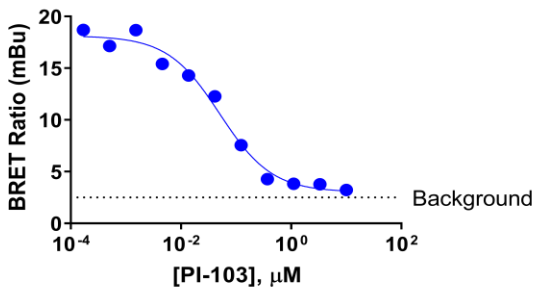


NanoLuc-PIK3CA(H1047Y)/PIK3R1 Optimizing [Tracer K-3]



	0.25µM	0.13µM	0.063µM	0.031µM	0.016µM
IC50	0.15	0.097	0.065	0.051	0.033

NanoLuc-PIK3CA(H1047Y)/PIK3R1 Example Compound Profiling



	PI-103
IC50	0.051

Example NanoBRET™ Tracer K-3 data in HEK293 cells transiently expressing NanoLuc®-PIK3CA(H1047Y)
Top Left Panel: Overview of the NanoBRET™ TE Assay. **Other Panels:** HEK293 cells were first transfected with NanoLuc®-PIK3CA(H1047Y) Fusion Vector and PIK3R1 Expression Vector (1 to 9 transfection ratio) and then were subsequently resuspended in OptiMEM prior to seeding into 96-well plates: **Top Right Panel:** Tracer affinity was measured by treating the cells with increasing concentrations of tracer in the presence or absence of a molar excess of unlabeled compound. **Bottom Left Panel:** The apparent cellular affinity of the unlabeled compound was measured at multiple fixed concentrations of the tracer, where the IC_{50} at the recommended tracer concentration is depicted in **orange** (4). **Bottom Right Panel:** An example compound profiling experiment at the recommended tracer concentration is provided.

Notes:

(1) This assay is run in the adherent format. See the **NanoBRET™ TE Intracellular Kinase Assay, Adherent Format** Technical Manual (#TM598) for protocol details.

(2) NanoBRET™ Tracer K-3 is supplied within the **NanoBRET™ TE Intracellular Kinase Assay, K-3** products (N2600, N2601, N2810). Additional assay components are supplied within these kits, including the NanoBRET™ Nano-Glo® Substrate, Extracellular NanoLuc® Inhibitor, and TE Tracer Dilution Buffer. Additionally, NanoLuc®-PIK3CA Fusion Vector (as a control) and Transfection Carrier DNA are provided in products N2600 and N2601. Transfection carrier DNA products (E4881 and E4882) are also available separately. For full details, please see the Promega website or technical manual for these products.

(3) Assay category is defined by the assay window at the recommended tracer concentration. It is detailed in section 7.A within the **NanoBRET™ TE Intracellular Kinase Assay, Adherent Format** Technical Manual (#TM598).

(4) See section 5 of **NanoBRET™ TE Intracellular Kinase Assay, Adherent Format** Technical Manual (#TM598) regarding approaches to achieve quantitative analysis of test compound affinity.

(5) NanoBRET™ TE Intracellular Assays have also been applied to Residence Time analysis. For a kinase example, please refer to Forster, M. *et al.* For an HDAC example please refer to Robers, M.B. *et al.*

References:

Vasta, J. D. *et al.* (2018) Quantitative, wide-spectrum kinase profiling in live cells for assessing the effect of cellular ATP on target engagement. *Cell Chem. Biol.* **25**, 206.

Robers, M.B. *et al*. (2015) Target engagement and drug residence time can be observed in living cells with BRET. *Nature Comm*. **6**, 10091.

Forster, M. *et al*. (2016) Selective JAK3 inhibitors with a covalent reversible binding mode targeting a new induced fit binding pocket. *Cell Chem. Biol.* **23**, 1335.