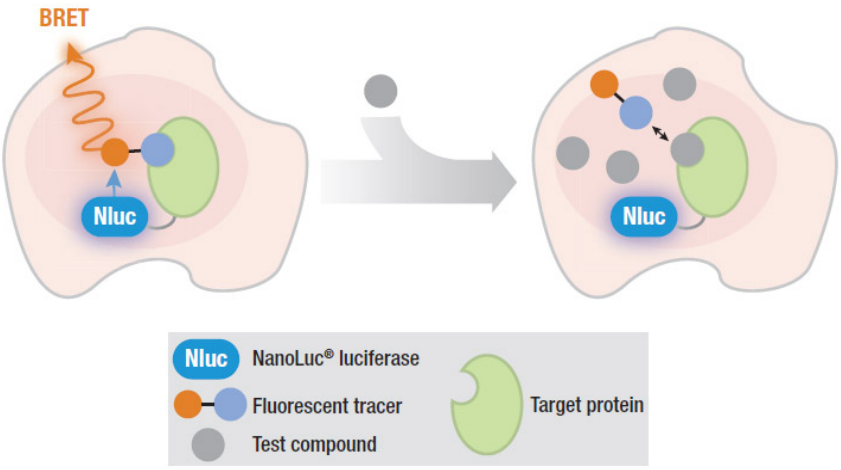
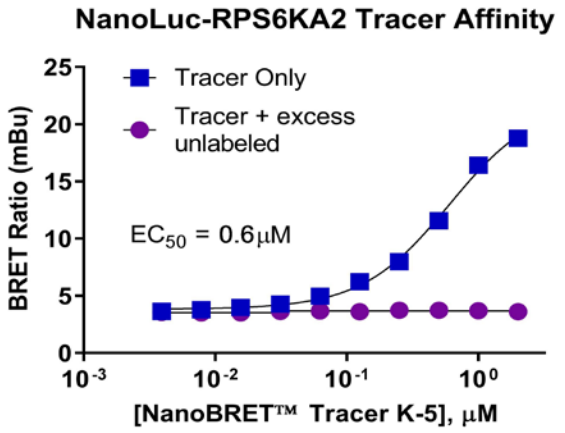
NanoBRET™ TE Intracellular Kinase Assay

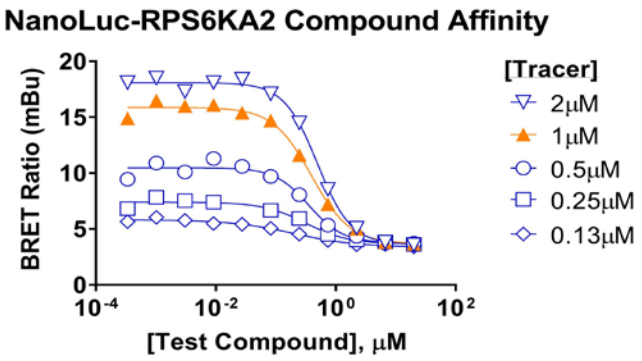
NanoLuc®-RPS6KA2 Fusion TE Assay

NanoBRET™ Tracer:	K-5 (1)
100X [Tracer]:	100µM in DMSO
Final [Tracer]:	1µM
Assay Category:	High Window (2)
Z':	0.82

Materials Needed	Cat. #
● NanoLuc®-RPS6KA2 Fusion Vector	● NV1991
● NanoBRET™ TE Intracellular Kinase Assay, K-5	● N2500, N2501, or N2530 (1)







	2µM	1µM	0.5µM	0.25µM	0.13µM
IC50	0.5	0.39	0.36	0.31	0.2

Representative data of NanoBRET™ Tracer K-5 competition in HEK293 cells transiently expressing NanoLuc®-RPS6KA2
Top Panel: Overview of the NanoBRET™ TE Assay. **Bottom Panels:** HEK293 cells were first transfected with NanoLuc®-RPS6KA2 Fusion Vector and then were subsequently resuspended in OptiMEM prior to seeding into 96-well plates: **Bottom Left 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 Right Panel:** The 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 (3).

Notes:

1) NanoBRET™ Tracer K-5 is supplied within the **NanoBRET™ TE Intracellular Kinase Assay, K-5** products (N2500, N2501, or N2530). Additional assay components are supplied within these kits, including the NanoBRET™ Nano-Glo® substrate, Extracellular NanoLuc® Inhibitor, tracer dilution buffer, and transfection carrier DNA. Additionally, BTK-NanoLuc® control vector is provided in products NV2500 and NV2501. For full details, please see the Promega website or technical manual for these products.
2) Assay category is defined by the assay window at the recommended tracer concentration. It is detailed in table 2 within the **NanoBRET™ TE Intracellular Kinase Assay, K-5** technical manual.
3) See section 5 of **NanoBRET™ TE Intracellular Kinase Assay, K-5** technical manual regarding approaches to improve quantitative analysis of test compound affinity.
4) 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:

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.

This protocol was developed by Promega Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM520, available at: **www.promega.com/protocols**