

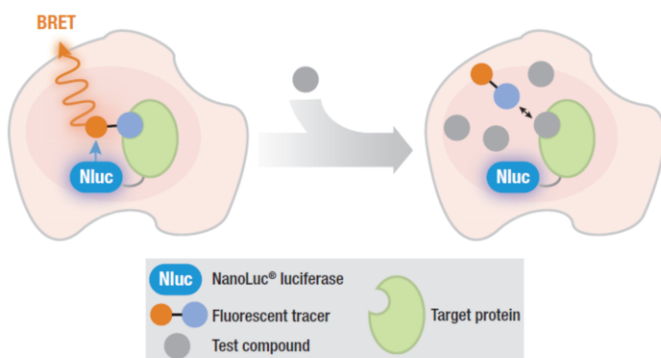
RIPK1 TE Assay

Assay Format:	ADH (1)
NanoBRET™ Tracer:	K-8 (2)
100X [Tracer]:	25µM in DMSO
Final [Tracer]:	0.25µM
Assay Category:	Medium Window (3)
Z':	0.84

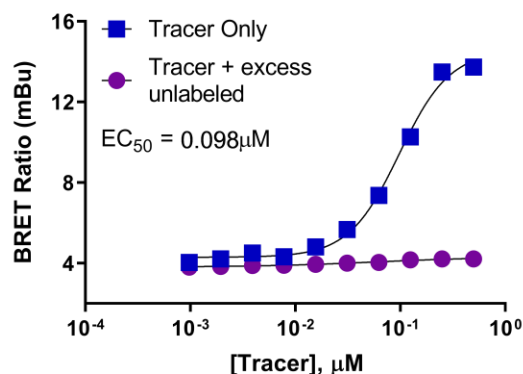
Materials Needed

- NanoLuc®-RIPK1 Fusion Vector (Cat.# NV4171)
- Transfection Carrier DNA (kit component; 2)
- NanoBRET™ TE Intracellular Kinase Assay, K-8 (Cat.# N2620, N2621, N2820; 2)

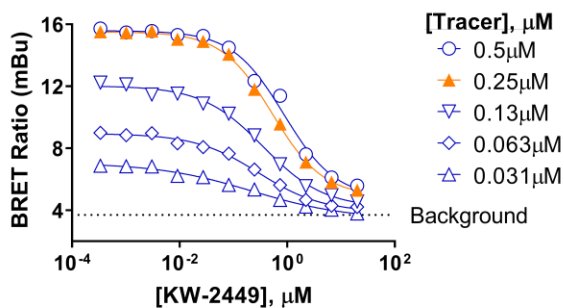
Overview of the NanoBRET TE Assay



NanoLuc-RIPK1 Tracer K-8 Dose Response

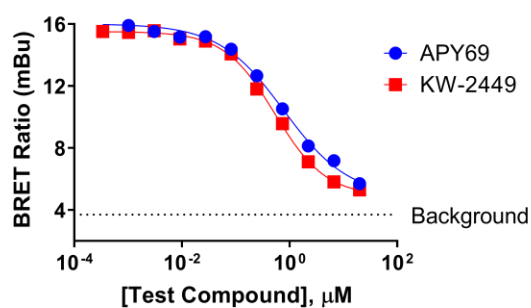


NanoLuc-RIPK1 Optimizing [Tracer K-8]



	0.5µM	0.25µM	0.13µM	0.063µM	0.031µM
IC ₅₀	0.87	0.53	0.36	0.29	0.22

NanoLuc-RIPK1 Example Compound Profiling



	APY69	KW-2449
IC ₅₀	0.78	0.53

Example NanoBRET™ Tracer K-8 data in HEK293 cells transiently expressing NanoLuc®-RIPK1

Top Left Panel: Overview of the NanoBRET™ TE Assay. **Other Panels:** HEK293 cells were first transfected with NanoLuc®-RIPK1 Fusion Vector and Transfection Carrier DNA (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 IC₅₀ 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-8 is supplied within the **NanoBRET™ TE Intracellular Kinase Assay, K-8** products (N2620, N2621, N2820). Additional assay components are supplied within these kits, including the NanoBRET™ Nano-Glo® Substrate, Extracellular NanoLuc® Inhibitor, and TE Tracer Dilution Buffer. Additionally, NanoLuc®-GSK3B Fusion Vector (as a control) and Transfection Carrier DNA are provided in products N2620 and N2621. 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.

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 #TM598, available at: www.promega.com/protocols