

## **EPHA4-NanoLuc®** Fusion TE Assay

NanoBRET<sup>™</sup> Tracer: K-4 (1) **Materials Needed** Cat. # 100X [Tracer]: 10µM in DMSO • EPHA4-NanoLuc<sup>®</sup> Fusion • NV1241 Final [Tracer]: 0.1µM Vector ● NanoBRET<sup>™</sup> TE Intracellular **Assay Category:** High Window (2) • N2520, N2521, Z': 0.94 Kinase Assay, K-4 or N2540 (1) BRET Min Nluc Nluc NanoLuc® luciferase Target protein Fluorescent tracer Test compound EPHA4-NanoLuc Tracer Affinity EPHA4-NanoLuc Compound Affinity Tracer Only 60 150<sub>T</sub> [Tracer] **BRET Ratio (mBu)** Tracer + excess -**D**- 1μM **BRET Ratio (mBu)** unlabeled 40 ↔ 0.33µM 100  $EC_{50} = 0.037 \mu M$ 🔺 0.11μM -₩ 0.037µM 20 50 0 10-6 10-4 10<sup>-2</sup> 10<sup>0</sup> 10<sup>2</sup> [Test Compound], µM 0. 10<sup>-3</sup> 10<sup>-2</sup> **10**<sup>-1</sup> 10° 1μM 0.33<sub>µ</sub>M 0.11µM 0.037 µM 0.012µM [NanoBRET<sup>™</sup> Tracer K-4], μM IC50 0.0085 0.0035 0.0019 0.0014 0.0012

Representative data of NanoBRET<sup>™</sup> Tracer K-4 competition in HEK293 cells transiently expressing EPHA4-NanoLuc<sup>®</sup> Top Panel: Overview of the NanoBRET<sup>™</sup> TE Assay. Bottom Panels: HEK293 cells were first transfected with EPHA4-NanoLuc<sup>®</sup> 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<sub>50</sub> at the recommended tracer concentration is depicted in orange (3).





## Notes:

- NanoBRET<sup>™</sup> Tracer K-4 is supplied within the NanoBRET<sup>™</sup> TE Intracellular Kinase Assay, K-4 products (N2520, N2521, or N2540). Additional assay components are supplied within these kits, including the NanoBRET<sup>™</sup> Nano-Glo<sup>®</sup> substrate, Extracellular NanoLuc<sup>®</sup> Inhibitor, tracer dilution buffer, and transfection carrier DNA. Additionally, DDR1-NanoLuc<sup>®</sup> control vector is provided in products NV2520 and NV2521. 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<sup>™</sup> TE Intracellular Kinase Assay, K-4 technical manual.
- 3) See section 5 of **NanoBRET<sup>™</sup> TE Intracellular Kinase Assay, K-4** technical manual regarding approaches to improve quantitative analysis of test compound affinity.
- 4) NanoBRET<sup>™</sup> 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 #TM519, available at: www.promega.com/protocols