**EPHA2-NanoLuc® Fusion TE Assay**

**NanoBRET™ Tracer:** K-4 (1)

100X [Tracer]: 10µM in DMSO

Final [Tracer]: 0.1µM

Assay Category: High Window (2)

Z': 0.89

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**Materials Needed**

- EPHA2-NanoLuc® Fusion Vector
- NanoBRET™ TE Intracellular Kinase Assay, K-4
- N2520, N2521, or N2540 (1)

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Representative data of NanoBRET™ Tracer K-4 competition in HEK293 cells transiently expressing EPHA2-NanoLuc® Fusion Vector. Top Panel: Overview of the NanoBRET™ TE Assay. Bottom Panels: HEK293 cells were first transfected with EPHA2-NanoLuc® 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 IC50 at the recommended tracer concentration is depicted in orange (3).
NanoBRET™ TE Intracellular Kinase Assay

Notes:

1) NanoBRET™ Tracer K-4 is supplied within the NanoBRET™ TE Intracellular Kinase Assay, K-4 products (N2520, N2521, or N2540). 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, DDR1-NanoLuc® 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™ TE Intracellular Kinase Assay, K-4 technical manual.

3) See section 5 of NanoBRET™ TE Intracellular Kinase Assay, K-4 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.