NanoBRET™ TE Intracellular Kinase Assay

NanoLuc®-NEK3 Fusion TE Assay

NanoBRET™ Tracer: K-5 (1)
100X [Tracer]: 200µM in DMSO
Final [Tracer]: 2µM
Assay Category: High Window (2)
Z': 0.82

Materials Needed

- NanoLuc®-NEK3 Fusion Vector: NV1791
- NanoBRET™ TE Intracellular Kinase Assay, K-5: N2500, N2501, or N2530 (1)

![Diagram of BRET and NanoLuc-NEK3 Tracer Affinity and Compound Affinity](image)

Representative data of NanoBRET™ Tracer K-5 competition in HEK293 cells transiently expressing NanoLuc®-NEK3 Fusion Vector. The affinities were 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).
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.