NanoBRET™ TE Intracellular Kinase Assay

CDK4/Cyclin D1 TE Assay

Assay Format: ADH (1)
NanoBRET™ Tracer: K-10 (2)
100X [Tracer]: 25µM in DMSO
Final [Tracer]: 0.25µM
Assay Category: High Window (3)
Z': 0.79

Materials Needed
- NanoLuc®-CDK4 Fusion Vector (Cat.# NV2811)
- CCND1 Expression Vector (Cat.# NV2621)
- NanoBRET™ TE Intracellular Kinase Assay, K-10 (Cat.# N2640, N2641, N2840; 2)

Example NanoBRET™ Tracer K-10 data in HEK293 cells transiently expressing NanoLuc®-CDK4
Top Left Panel: Overview of the NanoBRET™ TE Assay. Other Panels: HEK293 cells were first transfected with NanoLuc®-CDK4 Fusion Vector and CCND1 Expression Vector (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.
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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-10 is supplied within the NanoBRET™ TE Intracellular Kinase Assay, K-10 products (N2640, N2641, N2840). Additional assay components are supplied within these kits, including the NanoBRET™ Nano-Glo® Substrate, Extracellular NanoLuc® Inhibitor, and TE Tracer Dilution Buffer. Additionally, LIMK1-NanoLuc® Fusion Vector (as a control) and Transfection Carrier DNA are provided in products N2640 and N2641. 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).


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:


Robers, M.B. et al. (2015) Target engagement and drug residence time can be observed in living cells with BRET. Nature Comm. 6, 10091.


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