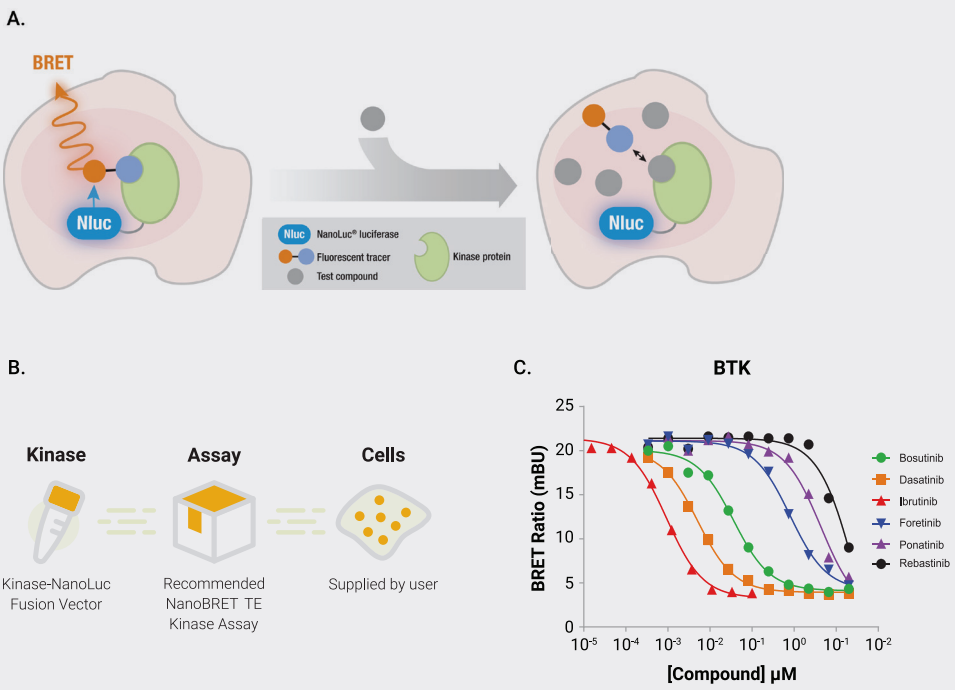
Kinase Target Engagement in Live Cells

The NanoBRET™ Target Engagement (TE) Intracellular Kinase Assay quantitates compound binding at select kinase protein targets within intact cells. This target engagement assay is based on the NanoBRET™ System, a bioluminescent energy transfer (BRET) technique designed to measure molecular proximity in living cells. Specifically, the assay uses competitive displacement between a test compound and a cell-permeable fluorescent NanoBRET™ tracer that reversibly binds to a NanoLuc® luciferase-kinase fusion protein expressed in cells. The NanoBRET™ TE Intracellular Kinase Assays and specific kinase-NanoLuc® luciferase fusion vectors are used together to measure kinase-compound affinity, occupancy, & residence time in live cells.

Features

- **Measure Kinase TE in Live Cells:** Test compound affinity & fractional occupancy quantified using direct competitive binding.
- **Use Full-Length Kinase:** Assays use full-length wild-type kinases, similar to native forms. Select mutant or domain specific kinase assays are also available.
- **Multi-Well Plate Format:** Simple, addition-only assay method, scalable from 96-well to 384-well or beyond.
- **Assays for over 300 Kinases:** Ready-to-use assays span the kinome, including RTKs & CDKs. Data & assay conditions for each kinase are provided.
- **Excellent Data Quality:** Ratiometric BRET data provides high reproducibility and low error.
- **Assess Residence Time:** Determine duration of test compound binding to a target kinase in live cells.

Figure 1. A NanoBRET™ Target Engagement Assay directly measures test compound affinity using a cell-permeable NanoBRET™ tracer

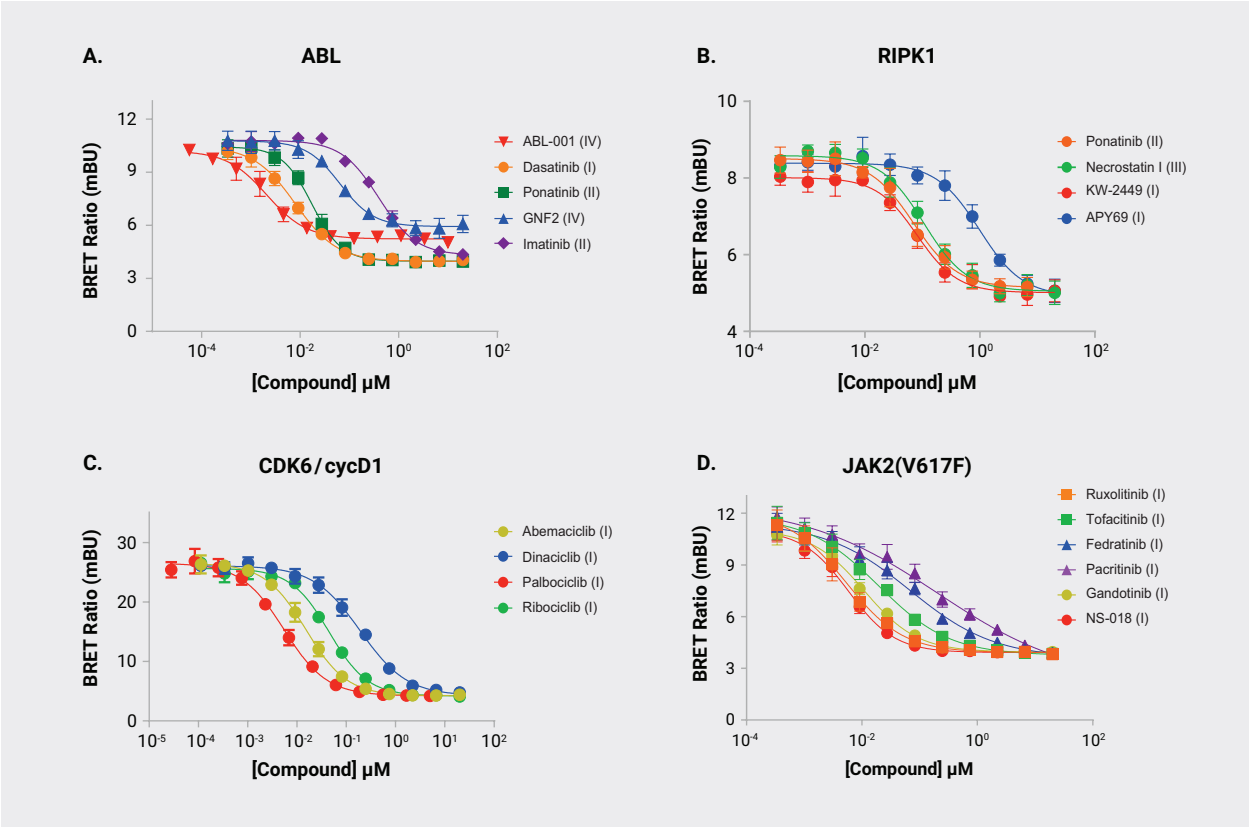


Panel A. Compound engagement is measured in a competitive format using a cell-permeable fluorescent NanoBRET™ tracer. Binding of the test compound to the NanoLuc®-tagged kinase results in a loss of NanoBRET™ signal between the target kinase protein and the tracer inside intact cells.

Panel B. Promega supplies the individual Kinase-NanoLuc® fusion vectors, NanoBRET™ TE Kinase Assays, and application notes for each kinase that details recommended assay conditions and data. You supply the cells and cell culture reagents.

Panel C. To determine test compound affinity, cells are treated with a fixed (recommended) concentration of NanoBRET™ tracer and titrated with varying concentrations of the test compound. Using the recommended K-5 tracer concentration for BTK-NanoLuc®, a series of inhibitors were tested for cellular affinity to BTK and the resulting dose response curves are shown.

Figure 2. Quantitation of intracellular affinity of kinase inhibitor types I-IV using NanoBRET™ TE



NanoBRET™ TE Intracellular Kinase Assays were used to quantify apparent cellular affinity of inhibitors with different binding mechanisms (i.e. type I, II, III, &/or IV inhibitors) to ABL1 (A), RIPK1 (B), CDK6/cyclin D1 complex (C) and JAK2(V617F) (D). The type of each inhibitor is denoted in parenthesis after the inhibitor name in each graph. ABL1, RIPK1, and CDK6 assays used wild-type kinases fused to NanoLuc. For the CDK6/cyclin D1 assay, cyclin D1 was co-expressed with NanoLuc-CDK6. JAK2 (V617F) assay used JAK2 with the V617F mutation.

For more information, visit: www.promega.com/NanoBRETKinaseTE

A list of available NanoLuc®-kinase fusion vectors and kinase-specific NanoBRET™ TE application notes are available at: www.promega.com/kinasevectors

Ordering Information

Product		100 Assays	1,000 Assays	10,000 Assays
NanoBRET™ TE Intracellular Kinase Assay, K-3	Cat. #	N2600	N2601	N2810
NanoBRET™ TE Intracellular Kinase Assay, K-4	Cat. #	N2520	N2521	N2540
NanoBRET™ TE Intracellular Kinase Assay, K-5	Cat. #	N2500	N2501	N2530
NanoBRET™ TE Intracellular Kinase Assay, K-8	Cat. #	N2620	N2621	N2820
NanoBRET™ TE Intracellular Kinase Assay, K-9	Cat. #	N2630	N2631	N2830
NanoBRET™ TE Intracellular Kinase Assay, K-10	Cat. #	N2640	N2641	N2840
NanoBRET™ TE Intracellular Kinase Assay, K-11	Cat. #	N2650	N2651	N2850

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