

## NanoBRET™ Target Engagement Assay for EPHB3 Kinase

**Cell line:** HEK293 human epithelial cell line from ATCC.

**Assay format:** 384-well plate format using Promega's NanoBRET™ TE Intracellular Kinase Assay.

**Assay protocol:** HEK293 cells transiently expressing NanoLuc®-EPHB3 Fusion Vector were seeded into the wells of 384-well plates. The cells were pre-treated with the NanoBRET™ Tracer K-4 and then treated with reference compound Dasatinib for 1 hour. The BRET signal was measured on an Envision 2104 Multilabel Reader. IC<sub>50</sub> value was calculated and IC<sub>50</sub> curve was plotted using the GraphPad Prism 4 program based on a sigmoidal dose response equation.

**Reference compound:** Dasatinib (IC<sub>50</sub> value): 6.687e-009 M

