

## NanoBRET™ Target Engagement Assay for TRKB 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®- TRKB Fusion Vector were seeded into the wells of 384-well plates. The cells were pre-treated with the NanoBRET™ Tracer K-5 and then treated with reference compound CTx-0294885 and Crizotinib 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:** CTx-0294885 (IC<sub>50</sub> value): 1.032e-007 M  
 Crizotinib (IC<sub>50</sub> value): 2.725e-007 M

