

## NanoBRET™ Target Engagement Assay for IRAK3 Kinase

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

**Assay format:** 384-well plate format.

**Assay protocol:** HEK293 cells transiently expressing IRAK3-NanoLuc fusion vector were seeded into the wells of 384-well plates. The cells were pre-treated with the tracer 05 and then treated with reference compound CTx-0294885 and Crizotinib for 1 hour. NanoBRET™ signal was measured on an Envision 2104 Multilabel Reader. IC50 (EC50) value was calculated and IC50 curve was plotted using the GraphPad Prism 4 program based on a sigmoidal dose-response equation.

**Reference compound:** CTx-0294885 (IC50 value): 6.001e-009 M  
 Crizotinib (IC50 value): 2.536e-009 M

