NanoBRET™ Target Engagement Assay for MAP4K1 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®- MAP4K1 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 for 1 hour. The BRET signal was measured on an Envision 2104 Multilabel Reader. IC$_{50}$ value was calculated and IC$_{50}$ curve was plotted using the GraphPad Prism 4 program based on a sigmoidal dose response equation.

**Reference compound:** CTx-0294885 (IC$_{50}$ value): 2.307e-007 M

Reference Compound IC$_{50}$ for MAP4K1

| HILLSLOPE | -1.037 |
| EC50      | 2.307e-007 |