## NanoBRET<sup>™</sup> Target Engagement Assay for EGFR[T790M, L858R]

## Protocol

HEK293 cells were transfected with EGFR(L858R, T790M)-NanoLuc® Iso1 Fusion Vector and seeded into the wells of 96-well plates. The cells were incubated with test compound for 2 hours following the addition of the NanoBRET<sup>TM</sup> tracer reagent. The BRET signal was measured on a luminometer after dispensing the NanoBRET<sup>TM</sup> Nano-Glo ® Substrate and Extracellular NanoLuc® Inhibitor into the wells. IC50 values were calculated by fitting the data to the following eaquation.

BRET ratio = Min + (Max - Min)  $\frac{1}{(1 + (IC50/[Compound])^{Slope})}$ 

