## NanoBRET<sup>TM</sup> Target Engagement Assay for CDKL5

## Protocol

HEK293 cells were transfected with NanoLuc®-CDKL5 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)  $\frac{1}{(1 + (IC50/[Compound])^{Slope})}$ 

