NanoBRET™ Target Engagement Assay for PKCη (PRKCH)

Protocol

HEK293 cells were transfected with PRKCH(PKCη)-NanoLuc® Fusion Vector and seeded into the wells of 96-well plates. The cells were stimulated with 1 μM PMA for 20 minutes and incubated with test compound for 20 minutes following the addition of the NanoBRET ™ tracer reagent. The BRET signal was measured on a luminometer after dispensing the NanoBRET™ Nano-Glo® Substrate and Extracellular NanoLuc® Inhibitor into the wells. IC50 values were calculated by fitting the data to the following eaquation.

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