**NanoBRET™ Target Engagement Assay for CDK4+Cyclin D1**

**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 co-expressing NanoLuc®-CDK4 fusion vector and Cyclin D1 Vector were seeded into the wells of 384-well plates. The cells were pre-treated with the NanoBRET™ Tracer K-7 and then treated with reference compound Palbociclib 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:** Palbociclib (IC50 value): 4.028e-009 M

![Reference Compound IC50 for CDK4+Cyclin D1](image)