



## **Compound Screening Protocol**

### **Day 0 Plate cells**

1700 cells per well in 384 well plate for most cell lines (MDA-MB-231, HS578T, SKBR-3, BXP-3). MCF10A-myc @ 500 cells/well. MiaPaCa-2 @ 1500 cells/well. MCF-7 @ 2500 cells/well. MDA-MB-453 @ 3500 cells/well. (50 uL/well for all cell lines)

### **Day 1 Add compound**

0.5 uL of compound or vehicle (DMSO) added to each well using Perkin Elmer EP3.

### **Day 4 Read (for 72 hr. time point)**

1. Aspirate 25 ul per well using Bio-Tek.
2. Add 25 uL ATP-lite/well using Thermo Multidrop Combi
3. Shake for 2 mins
4. Read on Analyst GT (Luminescence, Siquan 384)

Initial screen is generally done with compound concentrations of 1 uM, , on cells. Hits are screened at concentrations from 1 uM to approximately 0.5 nM