

Protocol for dilution and use of KCGS

96-well plate assay single concentration screen at 1 μ M

Step 1: Add to each well of the KCGS plate 9 μ L of DMSO (initial concentration 10 mM; final concentration 1 mM)

Step 2: Take 1 μ L from each well and transfer to a daughter plate in the desired screening format

Step 3: Add 9 μ L of media to each well of the daughter plate (initial concentration 1 mM; final concentration 100 μ M)

Step 4: Add 1 μ L of the liquid from Step 3 to the cell-based assay (100 μ L total assay volume) to generate a final concentration of 1 μ M of the inhibitor with a DMSO concentration of 0.1%.

These steps provide enough volume to perform an assay allowing for both technical and biological replicates as well as a counter screen in a control cell line.