Cell Number Count

Material:

HUVEC cell

JQ1

6 well plate (BD Falcon)

Procedure:

1. Seed the HUVEC cell at the density of $5x10^3$ cells/well
2. Add JQ1+ to the final concentration of 500nm into EGM2 growth medium
3. Digest the cell with Trypsin+0.25% EDTA and count the cell number with hemocytometer every day from Day2 after seeding
4. Change the medium every two days and stop the assay in 5-6 days.

Dose Test

Material:

HUVEC cell

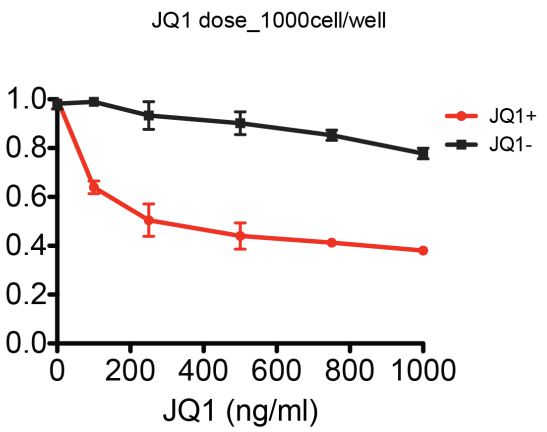
JQ1

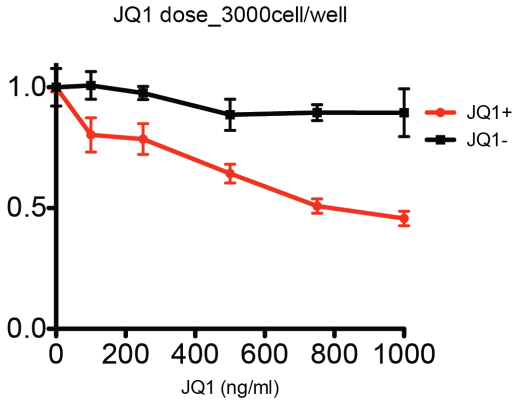
96 well plate (BD Falcon)

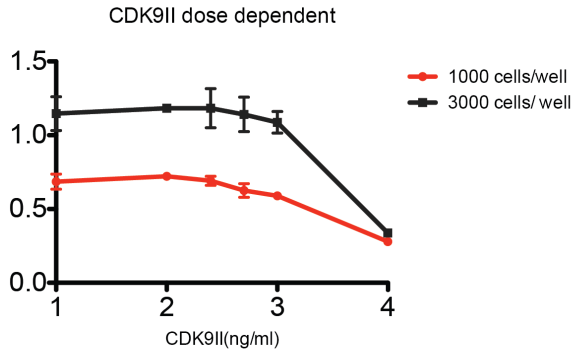
CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega)

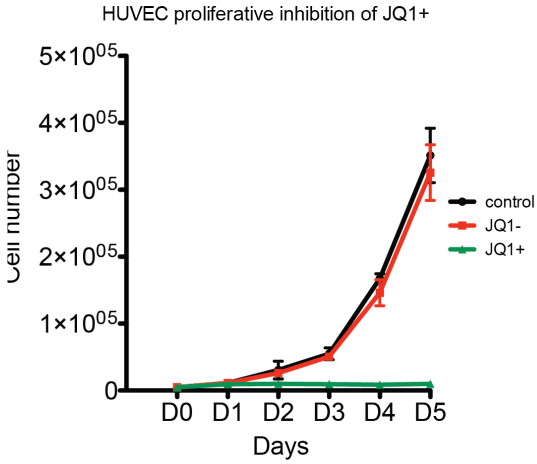
Procedure:

1. Seed the HUVEC cell at the density of $1x10^3$ cells/well
2. Add divergent dose of JQ1+ (0-1000nm) into EGM2 growth medium
3. Incubate the plate at 37℃ for 48–72 hours in a humidified, 5% CO2 atmosphere.
4. Add 20µl per well of CellTiter 96. AQueous One Solution Reagent.
5. Incubate the plate at 37℃ for 1–4 hours in a humidified, 5% CO2 atmosphere.
6. Record the absorbance at 490nm using a 96-well plate reader.
7. Plot the corrected absorbance at 490nm (Y axis) versus concentration of growth factor (X axis). Determine the X-axis value corresponding to one-half the difference between the maximum (plateau) and minimum (no growth factor control) absorbance values; this is the ED50 value (ED50 = the concentration of growth factor necessary to give one-half the maximum response.)


JQ1 dose_1000cell/well
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
JQ1 (ng/ml)
JQ1+
JQ1-


JQ1 dose_3000cell/well
1.0
0.5
0.0
0
200
400
600
800
1000
JQ1 (ng/ml)
JQ1+
JQ1-


CDK9II dose dependent
1.5
1.0
0.5
0.0
1
2
3
4
CDK9II(ng/ml)
1000 cells/well
3000 cells/ well


HUVEC proliferative inhibition of JQ1+
5×10^05
4×10^05
3×10^05
2×10^05
1×10^05
0
Cell number
D0
D1
D2
D3
D4
D5
Days
control
JQ1-
JQ1+