HNSCC Specfic Inhibitor Assay Protocol

Plan: cell line

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| Cell line:  DSN Here | Cells per well:  4000 | Plates used:  HNSCC Specific Vs 1 (plates 1-6) |

Total cells needed (minimum):

@2000 cells per well

X384 wells=768,000 cells per plate

X6 plates=4.6 x 10^6 cells

@4000 cells per well

X384 wells=1.536x 10^6 cells per plate

X6 plates=9.2 x 10^6 cells

@1200 (previous testing)

X384 wells=460,800 cells per plate

X6 plates=2.76 x 10^6 cells

@1800 cells per well (for poor growers)

X384 wells=691,200 cells per plate

X6 plates=4.2 x 10^6 cells

Concentration of cells:

Final in plate: 4000 cells per well = x cells x=160,000 cells/mL

25uL 1000uL

in 70mL = 11.2 x 10^6 cells (one 35mL tube for single agent plates with 5.6 million cells and another 35mL tube with 5.6 million cells for combination plates)

Media formula:

DMEM/F12 500mL

5% (Bovine Calf Serum) BCS 25mL

1x Anti-mycotic/Anti-bacterial 5mL

Protocol:

1. Wash cells with PBS to remove serum & media (10mL for one 100mm plate). 100mm confluent plate has approximately 5-10M cells depending on cell size.
2. Add 3ml trypsin and incubate at 37C for 10min (3mL for one 100mm plate), stop trypsinization with 10ml medium and pipette the cells up and down against the center of the plate to break up the clumps.
3. Filter all the cells into 50mL falcon tube using a sterile cell strainer, add 10mL medium to make the cell density lower and count cells

Cell counting:

50uL trypan blue + 50uL cell mix well and take 20uL out onto cell counting plate, don’t let the cover slip flow or the volume in the chamber changes. Count clear cells (blue cells are dead).

Cell density= average cell number in one square\*10^4 \* total number of mL

1. Spin down the cells and add the appropriate volume of base media to get 1million cells per mL of media. For example if your total cell count is 22.5 million cells add 22.5mL of base media and resuspend, then transfer 5.6mL of cell suspension into 29.4mL of base media to bring final volume to 35mL of media with 5.6 million cells total. You will need two tubes of 35mL with 5.6 million cells to plate all 6 inhibitor plates.
2. With the extra cells (if any) plate onto collagen coated coverslips at 100,000 cells per well for evaluation fo cell differentiation by vimentin & keratin immunofluorescence. If any additional left over, viably freeze those cells.
3. Put the two tubes on ice & bring to the tyner lab to use the automated plating system. Make sure to bring tubes for washing/cleaning automated plating system in tyner lab (diH2O (2 tubes) , 10%bleach, 70% EtOH, 0.05% Tween 20 in PBS, 1X PBS)
4. After plating cells in the tyner lab, incubate the plates for 72hrs.
5. Bring whatever cells are leftover from automated plating and put into a new 100mm plate to monitor cell growth & replating efficiency.
6. Plate MTS at 5uL per well after 72hrs
7. Incubate the MTS for 1-24 hrs as needed for appropriate color change.
8. Read plates using the photospectrometer (Pam’s lab behind Madelines bench) when color change is sufficient (usually between 1-5 hours but can sometimes be up to 24hrs – make sure to note time of MTS duration at plate reading) **[NOTE: we should be looking for final values in the untreated columns 8, 16 and 24 of between 0.2 and 1.0]**

Media needed:

MINIMUM

25uL per well

X384 wells=9.600mL per plate

X6 plates =57.6mL

**ACTUAL WORKING VOLUME NEEDED**

**70mL base media for 6 plates so you have some leftover at the end of the experiment to replate**

MTS needed:

2mL for each plate (5uL x 384 wells = 1920uL)

Total of 12mL for 6 plates

**Not Currently Using for HNSCC Specific Assay But for Future Reference:**

Dilution of gefitinib:

Stock concentration 10uM = 10,000nM

Final concentration of gefitinib: 50nM

Working concentration of gefitinib: 100nM

(make it double the target for 1:1 dilution from media already in the plates)

Calculations for gefitinib dilution:

(Mstock) (Vstock) = (Mtarget) (Vtarget)

Make 40mL of this to have extra:

(10000nM)(V) = (100nM)(40mL)

V = 0.40mL

39.60mL media + 0.40mL (10uM drug stock) = 40mL of 100nM stock