REAGENTS

PRODUCT	CATALOGUE	COMPANY
96 WELL PLATE	30096	HIMEDIA
ACRIDINE	TC262	HIMEDIA
ORANGE		
AGAROSE	A9539	HIMEDIA
ANTIBIOTIC	A002	HIMEDIA
B-27 SUPPLEMENT	17504-044	GIBCO
CALCIEN AM	C1430	INVITROGEN
DMEM	AL151A	HIMEDIA
HOECHST 33342	TC266	HIMEDIA
HUMAN EGF	#AF-100-15	PEPROTECH
HUMAN FGF	#100-18B	PEPROTECH
MEM	AL07S	HIMEDIA
MEM NEAA	ACL005	HIMEDIA
MitoView TM 633	70055-T	Biotium
MTT	102227	MP BIOMEDICALS
PLATES	70096	GENETIX
PROPIDIUM	TC252	HIMEDIA
IODIDE		
T25 FLASKS	70025	GENETIX
TEMOZOLOMIDE	SC-203292	CHEM CRUZ
TRYPSIN	TCL007	HIMEDIA

RECEIPES:

• MEDIA PREPARATION:

For 50 ml complete media: 40 ml DMEM/MEM in 50 ml falcon and add [1X] antibiotic, 10% FBS, maintain the volume to 50 ml with the media.

For 50 mL conditional media, 40 ml DMEM in 50 mL falcon and add [1x] antibiotic, [1x] B27 supplement, 20 ng/ml Human EGF, 20 ng/mL human FGF and maintain the volume to 50 mL.

• BEDDING WITH AGAROSE:

We used 0.9% agarose, for that dissolve 0.9 gm of agarose in 100 ml of autoclave PBS. To make a bed in 96 well plate, we used 50 uL agarose per well and it to solidify. Usually it takes 5-8 minutes to solidify.

• DRUG AND DYES:

TEMOZOLOMIDE (TMZ):

Stock: 10 mM dissolved in DMSO, working concentration 100 μM.

CALCEIN-AM:

Stock: 1M dissolved in DMSO, working concentration: 5 μM

PROPIDIUM IODIDE:

Stock: 10 μg/mL dissolved in PBS, working concentration: 10 μg/mL

HOECHST 33324:

Stock: 10 mg/ml dissolved in PBS, working concentration: 10 µg/mL

ACRIDINE ORANGE:

Stock: 10 mg/ml dissolved in PBS, working concentration: 10 µg/mL