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LD-HD Pigmentation Oscillator Model

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ABSTRACT

LD-HD pigmentation oscillator model uses B16 mouse melanoma cells which are seeded at a very low seeding density of 100 cells/cm^2 and are left without change in media. Cells under such conditions gradually starts pigmenting, beginning on Day 4 of LD. The cells survive up till day 12 of LD when they are trypsinised and reseeded at a higher density of 10000 cells/cm^2. Owing to high density culture, these cells start losing their pigment and gradually become depigmented by Day 16-20 i.e. four to eight days after seeding at high density. This is a great model which allows segregation of different stages of pigmentation and subsequent molecular changes to be followed over a period of 20 days.

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MANUSCRIPT CITATION:
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We use this protocol and it's working

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GUIDELINES

Cell Counting Guideline:

Number of cells in 1 quadrant= Number of cells present in 10^{-4}ml/0.1µl of media
(10^{-4} ml is actually the volume of hemocytometer quadrant.
{Each square width =250µm. so, for 4 squares in a quadrant}

i.e. 4 * 250µm =1000µm or 1mm
Length of each quadrant= 1mm
Width of each quadrant= 1mm
Height of hemocytometer chamber= 0.1mm
Volume of chamber = 0.1 mm^3 =10^{-4} ml
10^{-4} ml = 0.1µl

Number of cells in 1ul is therefore = 10 X Number of cells counted in one quadrant
Number of cells in 1ml is therefore = 10^4 X Number of cells counted in one quadrant

MATERIALS

1. Trypsin 0.1% (Gibco Invitrogen 2.5% **Cat no. 15090-046** diluted in Versene solution **Cat no. 15040066** to 0.1%)
2. 1x dPBS (Himedia **Cat no. TL1099-500ML**)
3. FBS (South American origin from Invitrogen **Cat no: 10270**)
4. DMEM High glucose (pH-7.4) prepared from DMEM powder **Cat no: D5648**. Reconstitute 1 bottle in 1litre of AMQ with 3.7 g of Sodium Bicarbonate. pH adjusted to 7.4, filtered and stored at 4°C.
5. B16 mouse melanoma cell line

SAFETY WARNINGS

1. For LD's the suspension should be diluted enough so that the number of cells per quadrant should be 80-100 only. More concentrated the suspension is, higher is the chance of error with every µl change in suspension volume.
2. For uniform distribution of cells, it is recommended to add the overall desired number of cells in the total MASTERMIX media and then distribute this media containing cells into flasks. Depending upon the number of flasks to set up; take the required number of cells (2500 or 7500 X total number of T25 or T75 required respectively). Add them to the mastermix media and distribute into flasks.

FOR LD (Low Density) CYCLE OF PIGMENTATION

1 Take a confluent B16 mouse melanoma cell culture maintained in DMEM+10%FBS media.
Confluency can be checked under a microscope and by change in color of media from pink to yellow-orange.

2 Discard media using a steri-pipette.

3 Wash the cells with 3-5ml of dPBS

4 Add 0.5-1ml of 0.1%trypsin solution to cover the entire area of flask base

5 Incubated for 2 minutes at 37^{o}C for cells to trypsinize

6 Meanwhile, prepare DMEM+10%FBS media (45ml DMEM +5ml FBS)

7 Take out the flask from incubator. Add 1ml of DMEM+ FBS (10%) to quench trypsin (Basically, double the volume of trypsin used).

8 Collect the suspension in a 15 ml falcon.

9 Centrifuge at 1000rpm for 7 minutes. Discard the supernatant and resuspend the pellet in few ml media.

10 Count the number of cells using hemocytometer.

11 Dilute the suspension if required. If the number of cells is less enough to be easily counted, no dilution is required.

12 For setting up an LD we require nearly 100 cells/cm^2 i.e. 2500 per 4-5ml media in a T25 flask or 7500 cells in 10-12 ml media in a T75 flask.

13 Take the required number of flasks and label them properly adding date and name of user, cell type, passage number.

14 Add 5ml-10 ml of DMEM (10% FBS) media containing cells (master mix cell suspension prepared above) into each T25 or T75 respectively.

15 Incubate the flasks at 37°C incubator under 5% CO_2.

FOR HD (High Density) CYCLE OF DEPIGMENTATION

16 Trypsinize the flasks on Day 12 of LD.

17 Seed the cells at a higher density of 10000 cells/cm 2 i.e 800000 cells per T75.
Similarly, 250000 cells per T25 for HD.

18 Change media on day 14 (if required).

19 Collect the pellet on Day 16 and Day 20 of LD.
Gradual depigmentation of cell pellet will be observed.