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Monoclonal Culture (Limiting Dilution) V.1

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COMMENTS 0

ABSTRACT

This protocol gives a step-by-step guidance on how to generate the homogenised gene-knockout (KO) monoclonal cell line from a polyclonal "KO" cells pool.

PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

This protocol is generated according to the protocol established and published from Addgene (https://www.addgene.org/protocols/limiting-dilution/)

KEYWORDS

Limiting dilution, Monoclonal cell culture, Single cell clone

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GUIDELINES

Prepare conditioned growth medium;
Cell counting and calculation;
Culture single cell suspension in 96-well cell culture plates;

MATERIALS TEXT

Stable polyclonal cell pool;
Fresh growth medium;
DPBS Buffer;
Trypsin;
Trypan Blue;

50 mL Falcon tubes;
0.2 µm syringe filter with syringe;
0.40 µm cell strainer mesh;
Hemocytometer;
Reagent Reservoir;
Multi-channel pipette (≥ 100 µL);
96-well cell culture plate(s);

BEFORE STARTING

Generate and prepare the stable polyclonal cell pool;

5m

Prep Conditioned Medium

1 Collect the used growth medium into a 50 mL Falcon tube.

Note

Do not collect the used growth medium if there is contamination or the medium turns orangish/yellowish.

2 Pipette the used growth medium through a 0.2 µm syringe filter to remove the waste product and dead cells.

3 Collect the supernatant (about 15 mL) used growth medium into a new 50 mL Falcon tube.
And then discard the waste product. 15 mL For three 96-well plates

Note

Collect 15 mL used growth medium if there are three 96-well cell culture plates ready for limiting dilution. Otherwise, collect 10 mL for two plates or 5 mL for one plate only.

4 Add fresh growth medium up to 30 mL (if there are three 96-well cell culture plates).
30 mL For three 96-well plates

Note

Roughly mix about 1:1 fresh and used growth medium. Add fresh medium up to 20 mL if there are two 96-well cell culture plates or up to 10 mL if there is only one plate.

5 Mix the medium thoroughly and seal the tube tightly for later use as the conditioned medium.

Prep the "KO" Cells

7m

6 Rinse cells with sufficient amount of DPBS Buffer.
15 mL

7 Aspirate the DPBS with aspirator.

2m

8 Trypsinise the cells with 1 mL of Trypsin in the culture incubator at 37°C for 2 min.
Trypsin-EDTA Solution **Sigma Aldrich Catalog #T-3924** 1 mL 00:02:00

9 Add 5 mL fresh growth medium to stop trypsinisation. 5 mL
Mix well.

10 Transport into a new 50 mL Falcon tube and spin at 1,000 rpm, 5 min. 5m
1000 rpm, Room temperature, 00:05:00

11 Discard the supernatant as possible using the aspirator.

12 Resuspend the cell pellet with 4 or 5 mL fresh growth medium. 4 mL For 1:8 passaging ratio
5 mL For 1:10 passaging ratio

13 Passage/Subculture cells at a ratio of 1:8 or 1:10 as usual.
Refer to the Cell Subculture protocol.

14 Pass the rest cell suspension into a new 50 mL Falcon tube through a 0.40 µm cell strainer mesh to break up cell clumps into individual cells.

Calculation

15 Pipette up and down couple times to make sure the cell suspension is evenly distributed.

16 Quantitate cell concentration using a hemocytometer.

Note

Count cells in replicates or even triplicates if necessary. Take the average of total counts to minimise the counting error.

17 Sample Calculation (Example Calculation)

17.1 Expect to have a cell concentration of ~3x10^6 cells/mL.

17.2 10X Dilution to make the concentration of ~3x10^5 cells/mL.
Count cells in replicates.

17.3 Expect to have the final concentration at ~5 cells/mL.
For 30mL conditioned medium, a total of ~150 cells are expected.
Thus, (~150 cells)/(~3x10^5 cells/mL) ≈ 0.5 µL of cell suspension is required.

17.4 Further dilute cell suspension in 1:100 DF to obtain an expected concentration of ~3x10^3 cells/mL.
(~150 cells)/(~3x10^3 cells/mL) ≈ 50 µL of cell suspension is required.

Monoclonal Culture Plate

18 Pipette 50 µL which carries ~150 cells from the cell suspension into the prepared 30 mL conditioned medium (Section 1).
Mix thoroughly.

19 Transfer the medium into a tray.

20 Use multi-channel pipette to transfer 100 µL into each well, leading to the cell concentration of ~0.5 cell/well.
100 µL

21 Place one control well containing ~1,000 cells in each plate.
In this case, pipette ~3 µL from the cell suspension of ~3x10^5 cells/mL (Section 17.2) into the control well and labelled.

22 Place the plates into the incubator until they are ready for single cell colonies screening.