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Isolation of single cells from adherent cell lines using Smart Aliquotor CE

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bhjmj4k6

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ABSTRACT

We present a method for the isolation of adherent cell lines using a microfluidic device. Using our protocol single cells can be rapidly isolated with minimal laboratory equipment and within biological safety cabinets for downstream single cell RNA sequencing. Step 1 to 6 is specific for adherent cell lines, optimise these steps for your cell culture system prior to following step 6 onwards.

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MATERIALS TEXT

Trypsin
RPMI media
PBS
Trypan Blue
Smart Aliquoter CE device (iBiochips: DHC-F01)
1.5ml Eppendorfs
15 ml Flacon tube
Haemocytometer (C-Chip - Labtech: DHC-F01)
PCR tubes or plate

1 Warm trypsin and RPMI media in a water bath before begining isolation protoccol

2 Remove media from cell culture flask and add trypsin. For a small fask (T25) add 3ml of trypsin.

3 Place cell flask into the incubator for 5 minutes

4 Observe cells under the microscope to check from detachment. Gently tap the side of the flask to assist with cell detachment.

5 Add an equal volume of media to the culture flask to neutralise the trypsin (eg 3ml for a T25 flask)

6 Transfer the contents of the flask to a 15ml falcon. Rinse the flask with a small amount of media and add to the falcon containing the cell suspension

7 Centrifuge cells at 500 xg for 5 minutes at room temperature

8 Discard the supernatant and resuspend the cells in media or PBS

9 Count the cells using trypan and determine cell viability using trypan blue. Take 10ul of cell suspension and add to 10ul of trypan blue. Mix thoroughly and add to a haemocytometer (C-Chip).

10 Dilute cells to a concentration of 100 cells/ul with a minimum final volume of 500ul

11 Carry out 3 serial dilutions as follows.

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Take 500ul of cell suspension and mix with 500ul of PBS in a 1.5ml Eppendorf. Pippette up and down to thoroughly mix. This tube should be at 50 cells/ul

11.1 Without changing the pipette tip, take 500ul of this dilution and add to a new eppendorf containing 500ul of PBS. Pipette up and down to thoroughly mix. This tube should be at 25 cells/ul

11.2 Without changing the pipette tip, take 500ul of this dilution and add to a new eppendorf containing 500ul of PBS. Pipette up and down to thoroughly mix. This tube should be at 12.5 cells/ul

11.3 Without changing the pipette tip, take 500ul of this dilution and add to a new eppendorf containing 500ul of PBS. Pipette up and down to thoroughly mix. This tube should be at 6 cells/ul

12 Count cells in triplicate using the haemocytometer as described above. Trypan is not necessary unless viability is of concern, as this will make counting the cells challenging due to the level of dilution

13 Cell count may vary from expected count due to the extreme dilution. Proceed with 1 or 2 further dilution steps, adjusted to account for the average cell count from step 12. The final concentration should reach 0.8 cells/ul and the volume of cells suspension transfered between serial dilutions should be 500ul.

14 Final cell concentration can be counted using a C-Chip (without trypan) although at least triplicate counts are required due to the stochastic nature of sampling at extreme dilutions

15 Ensure cells are uniformly suspended by mixing with a p1000

16 Using a p200, vertically inject 100ul of the final cell suspension into the central cap of the Smart Aliquotor CE device.

16.1 Maintain pressure on the pipette plunger as the full volume dispenses into the device. The channels of the device should become less visible as they fill with liquid.

17 Once the full volume has dispensed, hold the device and tilt the pipette to remove the cap from the device without releasing pressure on the pipette plunger. The centre cap should easily detach from the device.

18 Observe the device under a microscope at 4x magnification. Wells should be visible, as well as well location co-ordinates. Scan the device and record the co-ordinates of wells containing a single cell.

19 Using a pipette, transfer the contents of wells indicated to have a single cell into a PCR plate or PCR tube. If possible, transfer cell into a tube already containing mastermix or PBS to enable pipetting up and down to facilitate cell transfer

Once all single cells have been transferred out of the device, return the device to the microscope and observe that no cell

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20 remains in the identified wells.

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