CULTURE PREPARATION FOR DISPENSING MAMMALIAN CELLS.

Uno Single Cell Dispenser™.

BACKGROUND.

This protocol has been developed specifically for HEK-293 cells. Modifications may be necessary for other cell lines.

MATERIALS.

- HEK-293 human embryonic kidney cells (ATCC CRL-1573™)
- Eagle's minimum essential medium (EMEM; ATCC #30-200 3) + 10 % fetal bovine serum (FBS; ATCC #SCRR-30-2020) + 0.1 % antibiotic-antimycotic (100x; Thermo Fisher Scientific #1524011)
- Trypsin-EDTA, 0.25 % (Thermo Fisher Scientific #25200056)
- Dulbecco's phosphate buffered saline, 1x without Ca2+ and Mg2+ (DPBS; Thermo Fisher Scientific #21-031-CV)
- T-25 cell culture flask (Thermo Fisher Scientific #156367)
- 5 ml round-bottom polypropylene test tubes with 35 µm cell strainer cap (Thermo Fisher Scientific #352235)

METHOD.

Preparing cells.

1. Remove flask from incubator
2. Check confluence. Pre-dispense confluence should be about 80 % coverage on a T25 flask
3. Aspirate media
4. Add 3 ml of PBS directly to the flask to rinse the cell monolayer. Pour off PBS. Repeat this wash step
5. Add 3 ml of trypsin-EDTA directly to flask. Rock back and forth to ensure it completely covers the cell monolayer
6. Incubate cell flask with trypsin-EDTA for 10 minutes at 37 °C
7. Remove flask from incubator and add 3 ml of complete media to neutralize trypsinization reaction
8. Using a serological pipette, pipette up and down 10 times to mix the cell suspension
9. Transfer 1 ml of cells to a sterile microcentrifuge tube
10. Determine cell viability and concentration using a cell counting method (automated cell counter, hemocytometry, etc.)

Resuspending cells.

1. Centrifuge cell solution for 5 minutes at 200 rcf (other cell lines may need more or less centrifugation based on size)
2. Decant media/supernatant by pipette without disturbing cell pellet
3. Resuspend in 1 ml of PBS. Note: PBS volume can be increased/decreased depending on desired concentration
4. Using a serological pipette, gently pipette up and down 5 times to disperse cell pellet

Filtering cell suspension to remove debris.

The filtration step is optional, dependent on your cell line, but is recommended to disaggregate clumps and remove debris.

1. Take cap off the polypropylene test tubes with 35 µm cell strainer cap
2. Pipette 1 ml of resuspended cells into tube
3. Put cap back on
4. Tilt tube so that cell fluid rolls to the capped end (up against the filter/mesh screen)
5. Using a new pipette tip, hold the pipette at a gentle angle to pull fluid through the mesh screen into the tip. Make sure not to pull fluid all the way up through the tip into the barrel of the pipette (i.e., sucking up cells into the pipette)

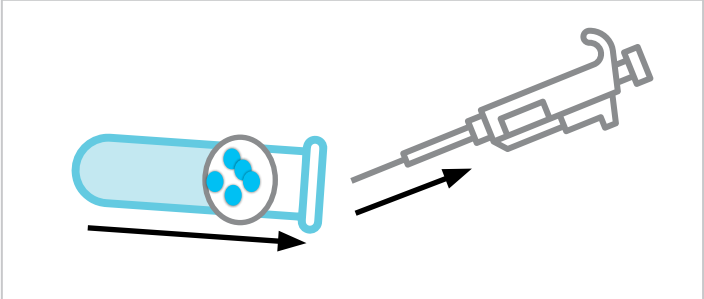

Figure 1

Diluting the sample.

1. Gently vortex resuspended sample for ~3 seconds before testing viability and concentration. Note: this concentrated cell suspension is your cell stock, retain this solution in case a new dilution must be made later
2. Dilute sample to target cell concentration (96-well plate: 100 cells/µl or 384-well plate: 200 cells/µl) using 1x PBS. This is your working cell suspension. *Note:* for best results, keep the working cell suspension at ambient conditions, and use within 10 minutes of dilution. Storage in incubator increases cell aggregation
3. Working cell suspension is now ready to be applied to the C1a dispensehead cassette

ADDITIONAL NOTES.

- Vortex gently for ~3 seconds each time before drawing up a sample
- Pipette the sample up and down 4-5 times from the bottom of the microcentrifuge tube to ensure a homogeneous suspension
- Aim to reduce the amount of time that cells sit before being dispensed. Using them within 10 minutes of preparing them, then discarding, is optimal

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