Adaptation to suspension culture

Subject:
Adaptation of cell cultures to suspension growth

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ADAPTATION OF CELLS FROM MONOLAYER TO SERUM-FREE SUSPENSION CULTURE

Typically, the adaptation of adherent cell lines to growth in suspension is carried out with the goal to achieve high cell densities and/ or growth in serum-deprived cell culture media.

The procedure described below is for cells grown in T-25 or T-75 flasks. About ten T-75 flasks provide enough cells (approximately 1-2 x 10^7) to seed a suspension culture in a spinner or shake flask.

For best results the following recommendations should be observed:
- Use cells 50% to 80% confluent
- Use cells with > 90% viability

CELL CULTURE MEDIA

Several basal cell culture media have been developed and optimized for suspension cell culture growth. We recommend the use of the following in the procedure described below:

- Minimum Essential Medium Eagle (MEM), Joklik's Modification for Suspension Cultures, for example Sigma refs 56449C, M0518 (powder) and M8028 (liquid). These media do not contain Calcium chloride. These basal media were designed for suspension culture systems. Calcium chloride has been omitted to reduce cellular attachment. References 56449C and M0518 are powder formulations and need the addition of Sodium Bicarbonate. M8028 is a ready-to-use liquid medium that requires the addition of L-glutamine.

- Dulbecco's Modified Eagle's Medium/ Ham's F-12 Nutrient Mixture without Ca++ and Mg++, e.g. Sigma reference D9785. This powder formulation does not contain L-glutamine, L-leucine, L-lysine, L-methionine, CaCl2, MgCl2, MgSO4, sodium bicarbonate, and phenol red. Needs to be supplemented with L-glutamine, Lleucine, L-lysine, L-methionine, sodium bicarbonate, and phenol red.

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This formulation is enriched with vitamins and amino acids compared to Joklik's MEM modification and contains also extra-buffering capacity (HEPES). This is the preferred formulation for the procedure described below.

To obtain the complete serum-free cell culture medium to be used in the procedure, supplement the basal cell culture medium of choice with 10% of Xerum-Free XF212 serum replacement, animal component-free, chemically defined.

PROTOCOL

1. Aspirate the growth medium from the flasks.
2. Add Phosphate Buffer Solution (PBS) without calcium and magnesium (5ml for T-25 flask or 15ml for T-75 flask). Leave for 5 min., then remove the washing solution.
3. - Add trypsin-EDTA solution to the side of the flask opposite the cell monolayer. Use the same volumes and procedure as for a normal cell passaging.
   - Incubate the flasks at 37°C for 5 to 10 min.
   - Monitor the cells during this incubation under a microscope.
   - When the cells round up, tap the flask to dislodge the cells.
4. Add growth medium (5ml for T-25 flask or 10 ml for a T-75 flask) to the cell suspension.
5. Add soybean trypsin inhibitor. Typically a 0.25 mg/ml trypsin inhibitor solution is used at the same volume as the trypsin-EDTA solution above.
6. Aspirate the cell suspension and transfer to a 15ml centrifuge tube. Centrifuge at 200×g for 4 min. Remove the supernatant.
7. Suspend the cells in 5 ml of growth medium. Centrifuge at 200×g for 4 min. Aspirate and discard the supernatant.
8. Resuspend the cells in 5ml of growth medium.
9. Perform a cell count with a trypan blue stain and estimate of the cell viability.
10. Dilute the cell suspension with growth medium such as to obtain a density of approximately 5 x 10^5 cells/ml.
11. Add Pluronic® F68 to a final concentration of 0.1% in order to protect the cells against shear stress. A 10% sterile cell culture tested solution is available from Sigma-Aldrich (reference P5556) – add 1ml of this solution to 100ml of cell suspension.
12. Transfer the cell suspension into sterile spinner or shake flasks. Leave enough headspace for adequate gas exchange (100 ml of cell suspension in a 250ml spinner flask or 250ml shake flask).
13. Loosen caps for gas exchange and place at 37°C in a humidified atmosphere of 5% CO^2. For spinner flasks, use an impeller speed of 75 to 95 rpm. For shake flasks, rotate on a shaker platform at 125 to 135 rpm.
14. Perform a daily count of viable cells and plot growth kinetics of the suspension culture.
15. When the cell density reaches 1×10^6 cells/ml (or on day 4 post-seeding) passage the cells at a density of 5 x 10^5 cells/ml. To perform this step aspirate the desired amount of cell suspension (e.g. take 50ml of a 1×10^6 cells/ml cell suspension) and dilute it with the same volume of fresh cell culture medium contai-ning 0.1% Pluronic F68 – this will lead to the suspension density of 5 x 10^5 cells/ml).
    NOTE: *if the cell density has not reached 1×10^6 cells/ml by day 4, the cells should be pelleted by centrifugation and resuspended in fresh medium containing Pluronic F68.*
16. Once cell density has reached 1×10^6 cells/ml with 90% viability by day 3 post-seeding for 3 passages, the cells are considered adapted to suspension culture. Seeding density can then be reduced to 2-3×10^5 cells/ml.