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Document # P-SFC 11/09
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Protocols for Converting Cells to Serum-Free Culture

Instructions for Use

Protocol #1: Medium Replacement

Approximate Time Required 2 weeks – 6 weeks
Culture Conditions:
Mammalian Cells 95% Air, 5% CO_2, 35ºC-37ºC
Invertebrate Cells Air, 25ºC-27ºC

Note: Adherent cell cultures that are exposed to trypsin during the subculturing process should be converted to serum-free growth using Protocol #2.

1. Begin with cultures at maximum cell density.
2. Split cells 1:2 using serum-free medium as the diluent.
3. Incubate cells until the maximum cell density is achieved.
4. Split cells 1:5 or to $3.0x10^5$ cells/ml for attachment independent cells or 30% confluency for attachment dependent cells using serum-free medium as the diluent.
5. Incubate cells until the maximum cell density is achieved.
6. If the cell viability is >85% at this point, and the generation time is similar to that observed with medium containing serum, the culture may be maintained on serum-free medium using a similar split schedule as originally optimized for medium containing serum.
7. If the cells exhibit slow growth or low viability, maintain the split ratio at 1:2 or 1:5 for three successive splits. The minimum cell density should be above $3x10^5$ cells/ml or 30% confluency during this period.
8. Gradually increase the split ratio to obtain a maximum value for the cell type being used.

Note: Some cells may require a small amount of serum for growth. If the cells have not adapted to serum-free cultivation using the above protocol, add 0.1%-0.5% serum to the culture or call Lonza Scientific Support at 1-800-521-0390.

Protocol #2: Serum Dilution

Approximate Time Required 2 weeks – 6 weeks
Culture Conditions:
Mammalian Cells 95% Air, 5% CO_2, 35ºC-37ºC
Invertebrate Cells Air, 25ºC-27ºC

1. Begin with cultures at maximum cell density.
2. Trypsinize adherent cultures and transfer to an appropriately sized centrifuge tube. Suspension cultures may be transferred directly to the centrifuge tube.
3. Sediment the cells using centrifugation at 350 x g for five minutes.
4. Resuspend the cells in serum-free medium containing 5% serum (V/V).
5. Adjust the cell concentration using the serum-supplemented serum-free medium to a maximum of $3x10^5$ cells/ml for attachment independent cells or a density to achieve not less than 30% confluency.
6. Plant the cells and incubate until a maximum level of cell density is achieved.
7. Repeat steps 2-6 using a lower concentration of serum at each split. We recommend beginning at 5% serum and lowering to 2%, 1%, 0.5% and finally 0.1% prior to eliminating serum from the culture.

Note: If the culture viability drops below 80% or if the generation time increases markedly following a decrease in the serum concentration, increase the serum level to the previous value and maintain the cells for two split cycles before lowering the level of serum again. It may be necessary to institute a more gradual decline in serum concentration with these cells. Some cells may require a small amount of serum for growth. If the cells have not adapted to serum-free cultivation using the above protocol, add 0.1%-0.5% serum to the culture or call Lonza Scientific Support at 1-800-521-0390.