MILIEUX SANS SERUM BIOWHITTAKER™ DE LONZA : PROTOCOLES D'ADAPTATION

Adaptation of Cell Cultures to Serum-free Medium

The conversion of a particular cell or cell line from growth in serum-containing medium to serum-free medium is achieved through the weaning process. However, weaning is not required for all cell types. Rapid conversion of a cell population to serum-free conditions can be achieved by pelleting the cells and resuspending them in the serum-free medium. While this may be successful for some types of cells, a gradual conversion is more likely to yield the desired result.

Weaning is actually a process by which a subpopulation of cells that can proliferate in the absence of serum is selected. The degree of difficulty in selecting these cells is a function of the physical and nutritional requirements of the cells and the complexity of the serum-free formulation. Conversion of cells to growth in UltraCULTURE™ Medium can be relatively simple because it is a complex formula. Other formulations may contain reduced amounts of protein (i.e., UltraCHO™ Medium and UltraDOMA™ Medium) or be entirely devoid of proteins and peptides (i.e., UltraDOMA-PF™ Medium). In practice, these formulations require slightly more attention during the weaning process. However, the benefits of a low protein serum-free growth environment and subsequent reduction in downstream processing procedures more than offset the extra time spent in the weaning process.

Maintenance of cellular function is an aspect of the weaning process that must be monitored. One needs to ensure that the subpopulation selected exhibits the same characteristics with respect to cellular function as the population that was cultivated in the presence of serum. These functions are diverse and may include receptor expression, viral susceptibility, monoclonal antibody production, and recombinant gene expression. In many cases, an increase in product yield has been noted when cells are converted to a serum-free environment. However, each investigator should monitor the cellular function of interest to their application during the weaning process.

We recommend two protocols for the conversion of cell populations to a serum-free environment. These protocols may be used for mammalian and invertebrate cell types. The first protocol may be used with attachment independent cells or cells that are loosely adherent and do not require trypsinization. It involves the gradual dilution of the serum-containing medium with serum-free medium. The second protocol may be used with both attachment dependent and independent cell types and begins with the serum-free medium supplemented with serum. A gradual reduction in the serum concentration is performed at each subculture until serum-free growth is achieved. This latter protocol has the added advantage of establishing the limit of serum concentration for the cell type. Some cells (especially transfected lines) require small amounts of serum (i.e., 0.1–0.5% v/v). This method allows the investigator to titrate the serum to the lower limit.

The two weaning protocols are presented on the following page. They represent our recommended procedures, however, each investigator may choose to make modifcations that better suit their particular application. In our experience, the minimum cell density maintained during the conversion process has a major effect on the outcome. We recommend that the cells be maintained above 3.0×10^5/ml for attachment independent and above 30% confluency for attachment dependent cells.

Protocols for Weaning Cell Cultures

Protocol #1: Medium Replacement – for Adherent Independent Cells (suspensions)

Approximate time required: 2 weeks – 6 weeks

Culture conditions:
- Mammalian cells: 95% air, 5% CO_2, 35°C–37°C
- Invertebrate cells: air, 25°C–27°C

1. Begin with cultures at maximum cell density.
2. NOTE: Attachment dependent cells that are exposed to trypsin during subculturing should be converted to serum-free growth using Protocol #2.
3. Split cells 1:2 using serum-free medium as the diluent.
4. Incubate cells until the maximum cell density is achieved.
5. Split cells 1:5 or to 3.0×10^5 cells/ml for attachment independent cells or 30% confluency for attachment dependent cells using serum-free medium as the diluent.
6. Incubate cells until the maximum cell density is achieved.
7. If the cell viability is >85% at this point, and the generation time is similar to that observed with serum-containing medium, the culture may be maintained in serum-free medium using a similar split schedule as originally optimized for serum-containing medium.
8. If the cells exhibit slow growth or low viability, maintain the split ratio at 1:2 or 1:5 for 3 successive splits. The minimum cell density should be above 3.0×10^5 cells/ml or 30% confluency during this period.
9. 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

Protocol #2: Serum Dilution – for Adherent Dependent Cells

Approximate time required: 2 weeks – 6 weeks

Culture conditions:
- Mammalian cells: 95% air, 5% CO_2, 35°C–37°C
- Invertebrate cells: air, 25°C–27°C

1. Begin with cultures at maximum cell density.
2. Trypsinize attachment dependent cultures and transfer to an appropriately sized centrifuge tube. Attachment independent cells may be transferred directly to the centrifuge tube.
3. Sediment the cells by centrifugation at 350 × g for 5 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 3.0 × 105 cells/ml for attachment independent cells or a density to achieve not less than 30% confluency for attachment dependent cells.
6. Plant the cells and incubate until a maximum 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 2 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 cell types may require a small amount of serum for growth. If the cells have not adapted to serum-free cultivation using the protocol described above, add 0.1–0.5% serum to the culture