

Adaptation of Cell Cultures to a Serum-Free Medium

Many cell lines are readily adapted to serum and protein-free media, while other more finicky cells have difficulty adjusting to the change and require a more specialized approach.

There are two methods available to adapt cells into a serum-free medium, depending on the characteristics of the cell line:

Sequential Adaptation/Weaning is a method where cells are eased into the new medium over a period time through a series of serum-reducing steps. This is the preferred method, and tends to be less harsh on the cells.

[Sequential Adaptation/Weaning Method-Method One](#)

Pass cells from the original medium sequentially through the following phases:

- Phase #1
75% Serum-supplemented Media/25% Serum-Free Media
- Phase #2
50% Serum-supplemented Media/50% Serum-Free Media
- Phase #3
25% Serum-supplemented Media/75% Serum-Free Media
- Phase #4
100% Serum-Free Media

[Sequential Adaptation/Weaning Method-Method Two](#)

1. Add serum to a small amount of the serum-free medium, at the same concentration present in the original medium. Pass cells from the original serum-containing medium into this mixture at a higher than normal cell density. Allow the cells one passage to adapt.

2. Slowly decrease the serum concentration, as Method #1, allowing the cells time to adapt at each stage.

3. Once the serum supplementation is decreased to zero, allow the culture several passages before using in assays or other manipulations.

Direct Adaptation is a method where cells are directly transferred from the serum-containing medium into the serum-free medium. Transfer a large volume of cells directly into the new serum-free or protein-free medium. Cells must be in mid-log phase with > 90% viability. Change approximately 50% of the volume the medium every 3-4 days to prevent it from turning acidic. Maintain a higher than normal cell density until the culture requires a daily medium replacement, at which time the culture may be expanded into multiple flasks.

Tips to Consider

During the adaptation process, cells are generally more sensitive to pH and temperature changes. Allow the cells at least one passage to adapt at each phase.

Cell Viability and Density It is critical that the culture be rapidly dividing in mid-log phase and greater than 90% viable when beginning the adaptation process. When splitting the culture, small splits of 1:2 or 1:3 are recommended to maintain the higher cell density while providing cell-produced growth factors that may assist the cells during the weaning process. As the culture adjusts to the new culture conditions, cell density will increase.

Cell Growth If the cells seem stalled at any point, allow them more time to adapt to the medium combination. The addition of 5mg/L insulin may then be decreased or completely eliminated once the cells have fully adapted into the new medium. Alternatively, adding a small amount of serum (1-3%) to the serum-free medium in Phase 4 of Method #1 may help the cells adapt to the new medium more readily. Then, slowly reduce the serum to zero over a few passages.

Suspension Cultures Mediatech's serum-free and protein-free media do not contain attachment factors found in serum. Adherent cultures will, over a period of passages, begin to lift off the surface and grow as a suspension line. Cells may also undergo morphological changes, as a result of the adaptation. Monitor the culture's growth rate and viability and as long as these factors are consistent, any morphology changes should not be a concern.

Cell Clumping Cells tend to clump together during adaptation to serum-free media. Gently triturate the clumps to break them up when passaging. Changing from static cell culture flasks to spinning erlenmeyer flasks may help prevent the clumping.

Antibiotics The use of antibiotics during serum-free adaptation is not recommended as serum proteins tend to bind to the antibiotic. Without the presence of serum proteins to bind to the antibiotic level may be toxic to the cells.

Precautions Ensure there is an adequate supply of frozen cell stock in the serum-supplemented medium prior to starting the adaptation process. Maintain a flask of each phase throughout the process in case the cells do not survive the next phase.