Start using XerumFree?

TNCbio's XerumFree™ Serum Replacement has been designed so as to be used in the same way and at the same concentration as conventional cell culture sera. You will go through the same two steps as usual.

Preparation of your cell culture medium

Just add XerumFree™ to your basal cell culture medium at the same concentration that you used to employ your preferred serum, e.g. 10% FBS

Adaptation of the cells to serum-free medium

There are several ways that can be employed to adapt the cells to growth in the new serum-free environment:

Direct Adaptation

This is a method where cells are directly transferred from the serum-containing medium into the serum-free medium.

To do this, seed a large volume of cells directly into the new serum-free medium. Cells should be in mid-log phase with > 90% viability. Change approximately 50% of the volume of 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.

Sequential Adaptation or Weaning Method

Method One

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

Phase 1: 25% Serum-Free Media / 75% Serum-supplemented Media

Phase 2: 50% Serum-Free Media / 50% Serum-supplemented Media

Phase 3: 75% Serum-Free Media / 25% Serum-supplemented Media

Phase 4: 100% Serum-Free Media

Method Two

Add serum to a small amount of the serum-free medium, at the same concentration as in the original medium (e.g. 10%). Pass the cells from the original serum-containing medium into this mixture at a higher than normal cell density. Allow the cells one passage to adapt.

Slowly decrease the serum concentration, as described in Method one, allowing the cells time to adapt at each stage.

NB XerumFree™ serum replacement does not contain any trypsin inhibitors nor deactivators. Therefor it is important that while using XerumFree™ attention is paid to:

- either use swift timing while passing the cells with trypsin. Prolonged trypsin contact may harm the cells. Cell pellets should be washed once with fresh medium and then be pelleted again;

- or use a soybean trypsin inhibitor;

Technical suggestions and considerations

The following counts for each of these methods, including the direct adaptation method:

Once the serum supplementation is decreased to zero, allow the culture several passages before using in assays or other manipulations. The reason is that the adaptation process is not immediate and it takes several passes for the cell line to stabilize.

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 is rapidly dividing in the 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. In certain cell types, this may also provide cell-produced (endogenous) 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.

Suspension Cultures: TNCbio's XerumFree™ serum replacements contain only a small amount of attachment factors as compared with those found in serum. Adherent cultures may therefore, over a period of passages, begin to lift off the surface and grow as a suspension

line. In order to avoid this, the use of a pre-coating stage with attachment factors/products may be recommended. The use of Pronectin F, for example, or poly-lysine, are efficient ways to avoid the cell detachment or bad cell adhesion. The coating with a small amount of serum is also an effective way to enhance cell adhesion, but TNCbio does not recommend this procedure, as it represents a setback to the completely serum-free principle.

Cell Clumping: Cells may tend to clump together during their adaptation to serum-free conditions. Gently triturate the clumps to break them up when passaging.

Antibiotics: The use of antibiotics during the XerumFree™ adaptation is not recommended. In the presence of serum, serum proteins tend to bind to the antibiotic. However, without the presence of serum proteins to bind to, the antibiotic level may become toxic to the cells.

From the moment the cells are adapted to XerumFree™, TNCbio recommends only in those cases the use of some antibiotics is considered really necessary, to use some low toxicity antibiotic like gentamycin instead of the conventional streptomycin/penicillin cocktail, generally used in serum containing media. The recommended use in XerumFree™ cell culture applications is 50 mg/L.

Precautions: If possible, make sure there is an adequate supply of frozen cell stock prior to starting the adaptation process. Also, maintain a flask of each phase throughout the process in case the cells do not survive the next phase.

Many cell lines are readily adapted to XerumFree™ media, while other more fastidious cells have difficulty adjusting to the change and require a more specialized approach.

TCNbio's cell culture experts will be happy to consult you during your XerumFree™ experience, so do not hesitate to contact us regarding any questions you may have or problems you may encounter. We will be happy to assist you !