Serum-free and Care-free? Not Quite

Serum-supplemented media (SSM) can present practical disadvantages, such as the presence of inhibitors and/or stimulants of growth/cellular functions or the difficulty/increased cost of purifying product. Serum-free media (SFM) can provide a more defined, controlled cell culture environment. However, unfamiliarity with SFM raises a number of technical questions. Stephen Gorfien, of Life Technologies, has attempted to answer some of them here.

Q. *How serum-free is "serum-free" media?*

A. "Serum-free" does not necessarily mean "protein-free" or "chemically defined." SFM may contain serum fractions or other proteins. Consult your medium vendor's technical services department if there are concerns of a scientific or regulatory nature about specific components.

Q. *How do I choose the appropriate SFM for my cells?*

A. There is no "universal" SFM applicable to all cell types under all culture conditions. There are, however, numerous commercially available SFM optimized for a variety of cell-specific applications. Choose a supplier with a reputation for quality and, preferably, one with a technical services department to answer specific questions you may have. Alternatively, you can make your own medium by following the SFM formulations developed for specific applications described in the literature, but be aware that you may not achieve the same results as the original authors because of differences in component quality and availability (especially water), order of component addition, and filtration scheme. A third option could be to use either a commercially available or published formulation as a starting point and then optimize to suit your particular application. Unless you have access to analytical instrumentation, however, this can often only be achieved through painstaking trial and error and may not be cost effective.

Q. *How do I switch my cultures from serum supplemented medium to SFM, or from one SFM to another?*

A. It may be possible to transfer your cells directly from serum supplemented medium into SFM, but in most cases sequential adaptation is better. In either approach, the starting culture must be healthy and active, with cell viability of at least 90%, and with the cells in the midlogarithmic phase of growth. The following sequential adaptation general protocol may be useful: Subculture the actively growing culture at twice the normal seeding density into a mixture of 75% serum supplemented medium (SSM) with 25% SFM. Allow culture to grow to normal passage density (monitor viable cell density daily to make sure cells are still actively dividing and have not reached normal peak density). Repeat the above two steps in progressively serum-free media, again using twice the normal seeding density. A typical progression would be 50:50 SSM:SFM, 25:75, and then 0:100. I would recommend keeping a backup culture in the previous SSM:SFM mixture until the cells have adapted to each new mixture.

Once the culture has been successfully passaged at least three times in 100% SFM, it can be considered SFM-adapted; the seeding density may then be returned to normal.

Q. *How do I purify my secreted product from spent SFM?*

A. In general, purifying protein products from SFM is easier than from serum supplemented medium if only because the protein content of SFM is lower, or at least better defined, than that of SSM. The protocol of choice will vary with properties of the secreted (or nonsecreted) product, the potential for contaminants, and the level of purity desired.

Q. *Are protease inhibitors necessary in a serum-free system?*

A. In the absence of serum, cells, and the secreted product, may be more susceptible to proteolytic degradation. In many cases, the effects of proteases may be minimized by optimizing growth and production kinetics to avoid extended periods of exposure to the enzymes. Addition of 1% bovine serum albumin (BSA) may buffer the action of proteases. However, protease inhibitors may still be necessary. If used, they should be carefully titered to determine the concentration that prevents degradation yet has minimal inhibitory effects on the cells. Note that protease inhibitors have a short half-lives once added to the medium and may themselves be toxic to cells.

Q. *Do I need to add growth and/or attachment factors to SFM?*

A. With the variety of SFM available for suspension and anchorage-dependent cells, it is impossible to generalize about the need for growth and attachment factors. Always read the product insert which accompanies commercially available SFM. Some SFM are sold complete and ready to use; others require supplementation with specific growth or attachment factors just prior to use (growth factors tend to have short half lives once added to medium, even when stored at 4°C). In some cases, the attachment factor must be used to coat the surface of the flask, rather than added directly to the SFM. If there is any doubt as to the applicability of a SFM or a supplement for a specific cell type, consult the medium manufacturer's technical services department.

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