

# ■ Products for Cell Biology

## SERUM-FREE CELL CULTURE MEDIA

### Using Quality Biological Serum-Free Media

It is the rare cell line or strain that can be removed from a serum containing cell culture medium and immediately grown and/or produce product in serum-free media. Invariably a period of adaptation is required. In most instances four passages will suffice. However, it may take as many as eight. The important point to note is that there are no hard and fast rules for adaptation. The following notes describe some approaches that can be used to help minimize the risk of failure.

### Hybridomas & Other Cells Growing in Suspension

#### “Weaning” Method

In this method, cells growing in serum containing media are sub-cultured into a 1:1 mixture of the original serum containing medium and the serum-free medium. At subsequent passages the proportion of the serum-free component of the growth medium is increased.

Example	% Serum Free Medium
First Pass	50
Second Pass	70
Third Pass	90
Fourth pass	100

The percentage of serum-free medium used at each step will depend on how well the cells proliferate in the preceding passage. The better the growth, the higher the percentage of serum-free medium that can be used at the following passage. Always retain one or more flasks grown in the same concentration of serum-free medium as the preceding passage.

#### “Quick” Method

Some cells can withstand being transferred from serum containing medium to 100% serum-free without a period of “weaning.” However, success using this method is more likely to be achieved if the cells are cultured at high density ( $5 \times 10^5$  -  $1 \times 10^6$  cells/ml) and left for 2 - 4 days before sub-culturing. Sub-culturing should be done at a split ratio of 1:2 to ensure successful adaptation.

In general terms, hybridomas with an NS-1 parent are easier to adapt to QBSF<sup>®</sup> media than those with a Sp2/0 parent.

### Anchorage Dependent Cells

Anchorage dependent cells can also be adapted to growth on serum-free media using the methods described for cells growing in suspension. However, there is the additional critical step of removing the cells from the surface on which they are growing. Under no circumstances should trypsin or other enzymes be used, since, in the absence of serum proteins, cells can be irreparably damaged.

To adapt anchorage dependent cells, the following technique is recommended. When the culture is 50 - 75% confluent, replace the serum-containing medium with either 100% serum-free medium (Quick Method) or a suitable mixture of serum-containing and serum-free medium. Once the culture is 90% - 100% confluent, remove the cells from the surface using ENZ-FREE<sup>™</sup> (Quality Biological, Inc.). This step can best be accomplished by washing the monolayer with magnesium- and calcium-free PBS, and adding sufficient ENZ-FREE to just cover the surface. After 10 - 20 minutes the cells should have rounded up and can be removed by firmly tapping the container. This step should be monitored carefully to avoid unnecessarily prolonged exposure to the ENZ-FREE reagent. Should you experience any difficulty with the adaptation process for your specific cells, or if you have any questions, please contact Customer Service at (800) 443-9331 or (301) 840-933, e-mail:

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