Adaptation of Cell Cultures to a Serum-Free Medium

There are two approaches to adapt cells to serum-free medium (SFM):

1. Direct adaptation — cells switched from serum-supplemented medium into SFM.
2. Sequential adaptation or weaning — cells switched from serum-supplemented medium into SFM in several steps.

   Sequential adaptation tends to be less harsh on cells than direct adaptation.

To adapt cells to SFM, it is critical that the culture is:

1. In mid-logarithmic phase
2. >90% viable
3. Seeded at a higher initial cell inoculum during adaptation

Below are general protocols for adaptation. Initial cell inocula, cell yields, and culture periods are intended as starting points for subsequent optimization.

Direct Adaptation

Some cells can be directly adapted from serum-containing medium to SFM. For direct adaptation, the cell inoculum should be 2.5×10^5 to 3.5×10^5 cells/ml. Subculture cells when the cell density is 1×10^6 to 3×10^6 cells/ml. Cells are fully adapted to SFM when the cell density is 2×10^6 to 4×10^6 cells/ml after 4 to 7 days in culture. Stock cultures of cells adapted to SFM should be subcultured in SFM every 3 to 5 days when the cell density is 1×10^6 to 3×10^6 with 90% viability.

Sequential Adaptation

Sequential adaptation is Invitrogen's preferred method for adapting cells to serum-free media (SFM), with a typical conversion being:

Passage 1
75% serum-supplemented medium: 25% SFM
Passage 2
50% serum-supplemented medium: 50% SFM
Passage 3
25% serum-supplemented medium: 75% SFM
Passage 4
100% SFM

Because the change from 75% to 100% SFM may be too stressful for your cells, you may need to carry the cells for 2–3 passages in a 10% serum-supplemented medium: 90% SFM mixture. Most cell lines can be considered fully adapted after 3 passages in 100% SFM. Occasionally you may have trouble getting your cells past a certain step even before going 100% SFM. If this happens, go back and passage the cells 2–3 times in the previous ratio of serum-supplemented media to serum-free media.

Points to Consider in Serum-Free Culture

Overall, cells in serum-free culture are more sensitive to extremes of pH, temperature, osmolality, mechanical forces, and enzyme treatment.

Antibiotics

It is best not to use antibiotics in serum-free media. If you do, we recommend that you use 5- to 10-fold less than you would in a serum-supplemented medium. This is because serum proteins tend to bind a certain amount of the antibiotic added; without these serum proteins the level of antibiotic may be toxic to certain cells.

Higher Density

Cells must be in the mid-logarithmic phase of growth with viability >90% prior to adaptation. Sequential adaptation may be necessary. Seeding cultures at a higher density than normal at each passage during SFM adaptation may help the process. Because some percentage of cells may not survive in the new culture environment, having more cells present will increase the number of viable cells to further passage.

Clumping

Cell clumping often occurs during adaptation to SFM. We recommend that you gently triturate the clumps to break them up when passaging cells.

Morphology

It is not uncommon to see slight changes in cellular morphology during and after adaptation to SFM. As long as doubling times and viability remain good, slight changes in morphology should not be a reason for concern.

Adaptation with Conditioned Medium

An alternate method for adaptation to SFM involves the use of "conditioned medium." This is medium the cells have been growing in for one full passage. If you choose this method, you can facilitate adaptation as follows:

Passage 1
100% serum-supplemented medium
Passage 2
50% medium from passage 1: 50% SFM
Passage 3
50% medium from passage 2: 50% SFM
Passage 4
50% medium from passage 3: 50% SFM
Passage 5
100% SFM

Whichever adaptation method you choose, we strongly recommend that you always take these precautions:

- Make a frozen stock of the cells in the serum-supplemented media prior to adaptation.
- Keep a culture going of the cells in each prior condition when starting the next level of adaptation as a fall-back if the cells do not survive in the next passage.