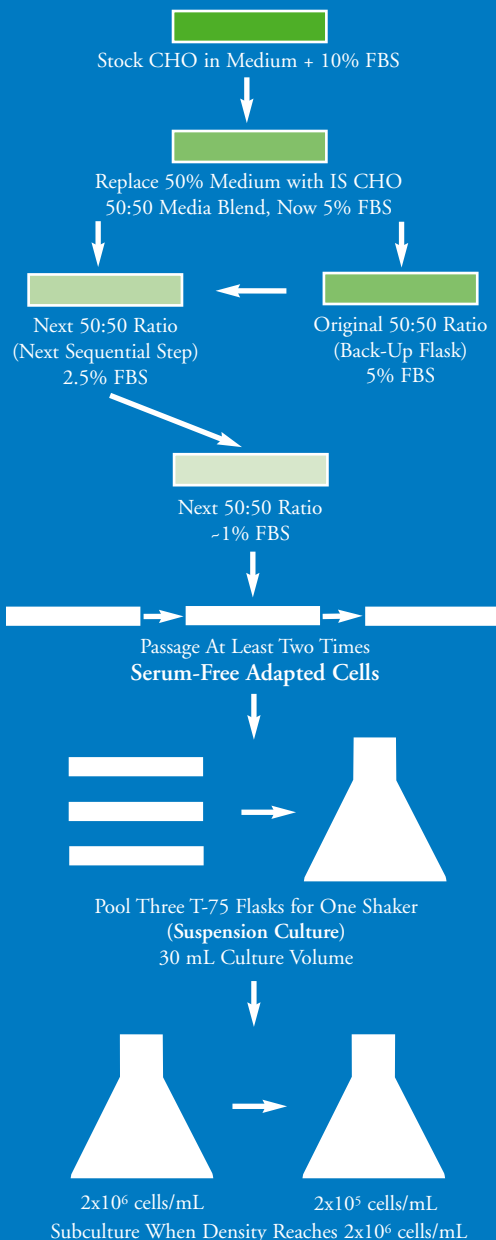




## Sequential Adaptation Protocol



IS CHO™ is provided without L-Glutamine to extend shelf life and allow the use of L-Glutamine feeding strategies. This medium may be supplemented with 8mM L-Glutamine.

The following adaptation steps are recommended for optimum medium performance.

### Direct Adaptation from Serum-Supplemented Media to Serum-Free IS CHO

- Cells can be grown as adherent monolayers and as non-adherent suspension cultures.
- Cells grown in serum-supplemented (5% - 10% FBS) or serum-free medium can be directly subcultured into serum-free IS CHO medium. It is critical that cells be in the mid-logarithmic phase of growth with at least 90% viability.
  - Adherent monolayers can be subcultured directly into serum-free IS CHO in:
    - Adherent culture (T-25, T-75, etc) at an initial density of 1:5 or
    - Suspension culture (e.g., 125 mL shaker flasks with 30 mL culture volume and shaking at 160 rpm) at an initial density of  $2 \times 10^5$  cells/mL.
  - Suspension cultures can be directly subcultured to  $2 \times 10^5$  cells/mL in serum-free IS CHO.
- Incubate cultures accordingly at 37°C and 5% CO<sub>2</sub> until the viable cell density exceeds  $2 \times 10^6$  cells/mL with a viability greater than 90%.
- Cells should be subcultured to  $2 \times 10^5$  cells/mL twice weekly when the viable cell density exceeds  $2 \times 10^6$  cells/mL, with viability greater than 90%.
- If the direct adaptation method gives suboptimal performance, use the sequential adaptation method.

NOTE: Adherent cells can be dislodged by scraping or by using trypsin. If trypsin is used, the cells should be centrifuged and washed once with PBS or HBSS to remove all traces of trypsin prior to resuspending and subculturing in serum-free IS CHO. We have successfully seeded cells grown in DME + 10% FBS from a nearly confluent T-75 flask (stationary culture) directly into 30 mL of serum-free IS CHO at an initial density of  $2 \times 10^5$  cells/mL in a 125 mL shaker flask (suspension culture).

### Sequential Adaptation from Serum-Supplemented Media to Serum-Free IS CHO

- Cells can be grown as adherent monolayers and as non-adherent suspension cultures.
- Cells grown in 5% - 10% FBS supplemented medium to a density of  $1-3 \times 10^6$  cells/mL should be subcultured to a density of  $2 \times 10^5$  cells/mL into a 50:50 ratio of serum-free IS CHO medium and the original serum supplemented medium.
- Incubate accordingly (for adherent or suspension cultures) at 37°C and 5% CO<sub>2</sub> until the viable cell density exceeds  $2 \times 10^6$  cells/mL and viability is greater than 90%.
- Subculture to a density of  $2 \times 10^5$  cells/mL into a 75:25 ratio of serum-free IS CHO medium and the original serum supplemented medium.
- When the viable cell density exceeds  $2 \times 10^6$  cells/mL, with greater than 90% viability, subculture to  $2 \times 10^5$  cells/mL into 100% serum-free IS CHO medium. Higher seeding densities ( $3-5 \times 10^5$  cells/mL) may be required for the first few passages in IS CHO only. Once cells have adapted to growth in IS CHO alone, lower seeding densities can be used for subculturing.
- Continue to subculture twice weekly when the viable cell density exceeds  $2 \times 10^6$  cells/mL. Continue passaging in IS CHO for several passages until the viable cell density exceeds  $2 \times 10^6$  cells/mL with greater than 90% viability. At this stage, the cells are adapted to IS CHO.
- In case the cells do not survive at any one particular stage of adaptation, maintain the cells for an additional passage in the previous stage medium ratio before subculturing into the next stage.

For more information on any of our Industrial Cell Culture Products, call 1 800 437 5706 and request that your Territory Manager contact you. Visit our website at [www.irvinesci.com](http://www.irvinesci.com) or e-mail us at [nucleus@irvinesci.com](mailto:nucleus@irvinesci.com).