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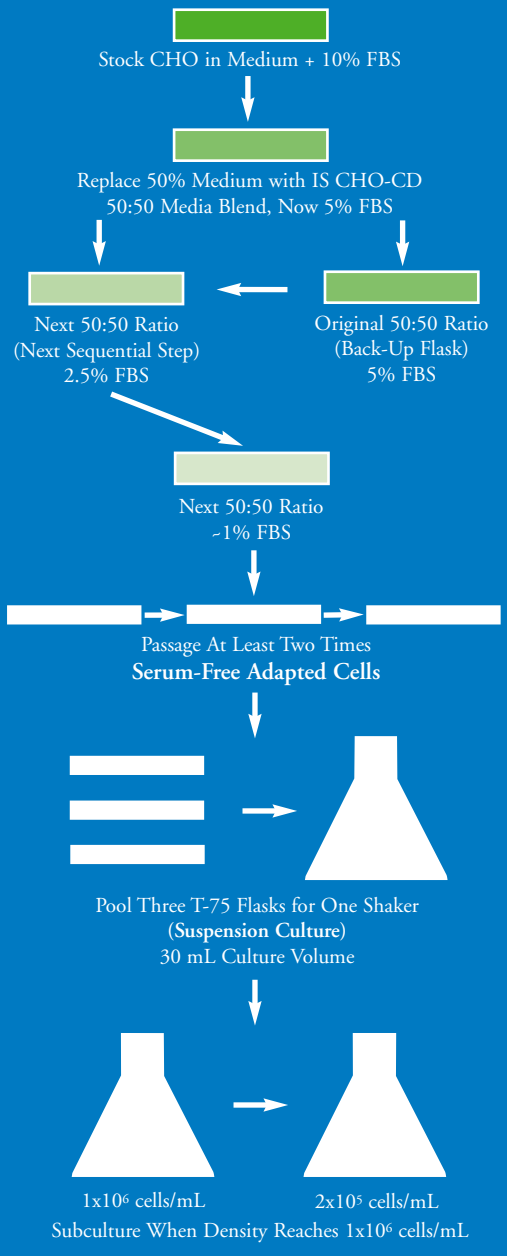
IS CHO-CD™ Adaptation Protocol

IS CHO-CD™ is provided without L-Glutamine to extend shelf life and to 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.

Sequential Adaptation Protocol



Direct Adaptation from Serum-Free CHO Media to IS CHO-CD

In many cases, CHO cells may be subcultured from a serum-free medium (e.g., IS CHO) directly into IS CHO-CD.

1. Dispense IS CHO-CD medium into an appropriate culture vessel and equilibrate to 37°C and 5% CO_2.
2. Passage CHO cells from serum-free culture into IS CHO-CD at 3 X 10^5 viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37°C and 5% CO_2 until the viable cell density reaches 1 X 10^6 cells/mL.
4. Subculture into fresh IS CHO-CD medium at 2 X10^5 cells/mL starting density.
5. Maintain cells in IS CHO-CD for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

Sequential Adaptation from Serum-Free Media to IS CHO-CD

Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-free medium and IS CHO-CD medium in a 1:1 ratio into an appropriate culture vessel and equilibrate to 37° and 5% CO_2.
2. Passage CHO cells from serum-free culture into the blended medium (Step I) at 3 X 10^5 viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37°C and 5% CO_2 until the viable cell density reaches 1 X 10^6 cells/mL.
4. Subculture at 3 X 10^5 cells/mL starting density into fresh medium prepared in a 1:3 ratio of original serum-free medium to IS CHO-CD medium.
5. Repeat steps 3 and 4 with sequential dilution ratios of 1:7, 1:15 and 0:1 of the original serum-free medium and IS CHO-CD. If the cells look unhealthy or the growth rate declines significantly at a particular step of adaptation, maintain the cells for an additional passage in the media ratio of the previous step before subculturing into the next ratio.
6. Maintain cells in IS CHO-CD for several passages, subculturing twice weekly to allow complete adaptation and ensure optimum performance.

Sequential Adaptation from Serum-Supplemented Media to IS CHO-CD

1. The direct transfer of cells from serum-supplemented media to IS CHO-CD medium is not recommended. Sequential adaptation can be achieved by gradual weaning of cell cultures from a serum-supplemented medium to IS CHO-CD medium.
2. Cells can be adapted to IS CHO-CD medium by gradually reducing the serum concentration using the sequential ratios of 1:1, 1:3, 1:7, 1:15 and 0:1 of serum-supplemented medium and IS CHO-CD medium. Cells should be grown and subcultured at the densities previously described in Section II (Sequential Adaptation from Serum-Free Media to IS CHO-CD) above.

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 or e-mail us at nucleus@irvinesci.com.

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