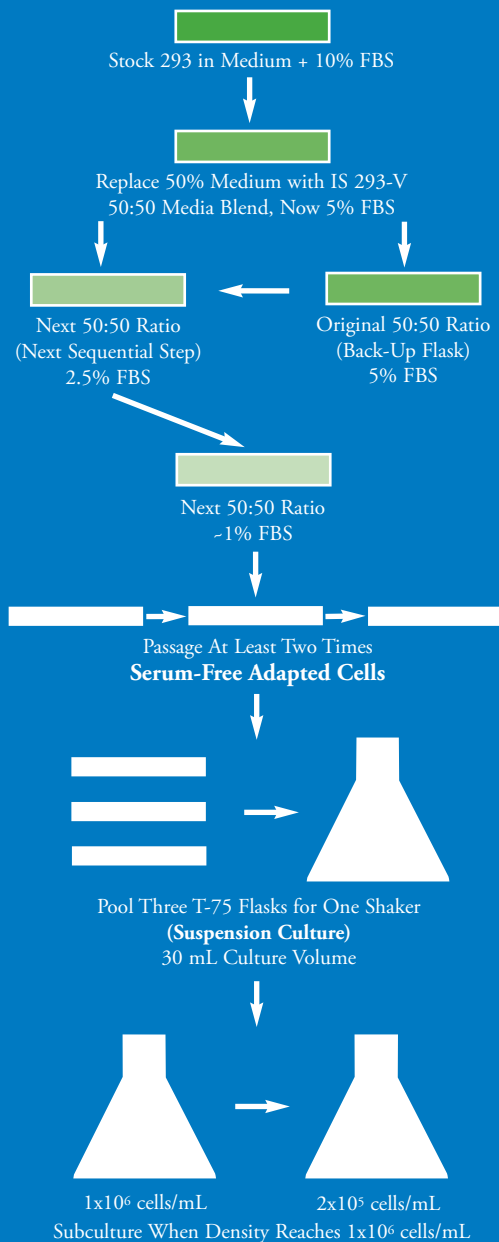




## Sequential Adaptation Protocol



Cells growing in serum-free conditions are more susceptible to changes in pH and toxic substances. Frequent monitoring is recommended.

The following adaptation steps are recommended for optimum medium performance.

### Direct Adaptation from Serum-Free 293 Media to IS 293-V™ Medium

293 cells adapted to growth in serum-free media may be subcultured directly into IS 293-V.

1. Dispense IS 293-V Medium into culture vessel and equilibrate to 37° C and 5% CO<sub>2</sub>.
2. If cells are grown under agitated conditions (eg. shaker flasks), IS 293-V should be supplemented with 0.1% Pluronic® F-68.
3. Subculture 293 cells from serum-free culture directly into IS 293-V at 3-5 x 10<sup>5</sup> viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 85% viability before passaging.
4. Incubate cultures at 37° C and 5% CO<sub>2</sub> (at 110-140 rpm for shaker culture) until the viable cell density reaches 1-2 x 10<sup>6</sup> cells/mL. Cultures should be fed every 3-4 days.
5. Subculture into fresh IS 293-V Medium at 3-5 x 10<sup>5</sup> viable cells/mL starting density.
6. Maintain cells in IS 293-V for several passages, subculturing once or twice weekly (as needed) to allow for complete adaptation and assure optimum performance. If sub-optimal performance is observed, sequential adaptation should be used.

### Sequential Adaptation from Serum-Free Media to IS 293-V Medium

1. Dispense the original serum-free medium and IS 293-V Medium in a 3:1 ratio into an appropriate culture vessel and equilibrate to 37° C and 5% CO<sub>2</sub>. For agitated suspension cultures (eg. shaker flasks), IS 293-V Medium should be supplemented with 0.1% Pluronic F-68.
2. Subculture the 293 cells from the original serum-free culture into the 3:1 (original: IS 293-V) at 3-5 x 10<sup>5</sup> viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 85% viability before passaging.
3. Incubate cultures at 37° C and 5% CO<sub>2</sub> (at 110-140 rpm for shaker flasks) until the viable cell density reaches 1-2 x 10<sup>6</sup> cells/mL.
4. Subculture the cells at a starting density of 3-5 x 10<sup>5</sup> viable cells/mL into the next sequential ratio (2:1) of original serum-free medium and IS 293-V.
5. Repeat steps 3 and 4 with sequential dilution ratios of 1:1, 1:2, 1:4 and 0:1 for the original serum-free medium and IS 293-V. 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 previous step before subculturing into the next ratio.
6. Maintain cells in IS 293-V for several passages, subculturing once or twice weekly to allow for complete adaptation and assure optimum performance.

### Sequential Adaptation from Serum-Supplemented Media to IS 293-V Medium

Direct transfer of cells from serum-supplemented medium to IS 293-V is not recommended. Adaptation to serum-free culture can be achieved with gradual weaning from serum-supplemented medium to IS 293-V Medium. This method allows for adaptation of 293 cells from adherent culture to serum-free suspension culture using stepwise reduction of serum.

1. Cells can be adapted to IS 293-V Medium by gradually reducing the serum concentration using the sequential ratios of 1:1, 1:3, 1:7, 0:1 of serum-supplemented medium and IS 293-V.
2. Dispense medium into culture vessel at a 3:1 ratio of serum-supplemented medium and IS 293-V, and equilibrate to 37° C and 5% CO<sub>2</sub>.
3. Harvest 293 cells from an established serum-supplemented culture at log phase density and high viability (at least 85%). **Trypsinization is not recommended since it may adversely affect the cells during this adaptation procedure.** Cells should be detached from the flasks surface without using trypsin by gently sloughing (pipetting the medium across the monolayer), scraping or rapping the flask sharply against your hand or a surface several times.  
**Note:** Cell aggregates can be dispersed into single-cell suspensions using a small bore pipette.
4. Continue to subculture weekly (as needed) when cell density reaches 1-2 x 10<sup>6</sup> cells/mL, to a starting density of 3-5 x 10<sup>5</sup> cells/mL. Cell growth rate may decline significantly when initially cultured in complete IS 293-V Medium, and may require a higher initial seeding density.
5. Culture cells for a minimum of 2 passages to assure complete adaptation.
6. Replace culture medium with fresh medium every 3-4 days. When cells are completely adapted to serum-free conditions they will become loosely adherent or non-adherent.
7. If the cells do not thrive, or look unhealthy at any one particular stage of adaptation, maintain the cells for an additional passage in the previous stage media ratio before subculturing into the next stage.
8. Once adapted to serum-free growth, the non-adherent cultures can be scaled up to shakers, spinners, and larger culture systems. For agitated culture conditions, IS 293-V Medium should be supplemented with 0.1% Pluronic F-68.
9. Under serum-free conditions, the amount of selective drug used (if any) should be decreased. The concentration should be determined for your specific cell system.

For more information on all 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).

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