IrvineScientific®
Grow With Us

IS BAC™ Adaptation Protocol

IS BAC™ 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.

Direct Adaptation from Serum-Supplemented Media to Serum-Free IS BAC

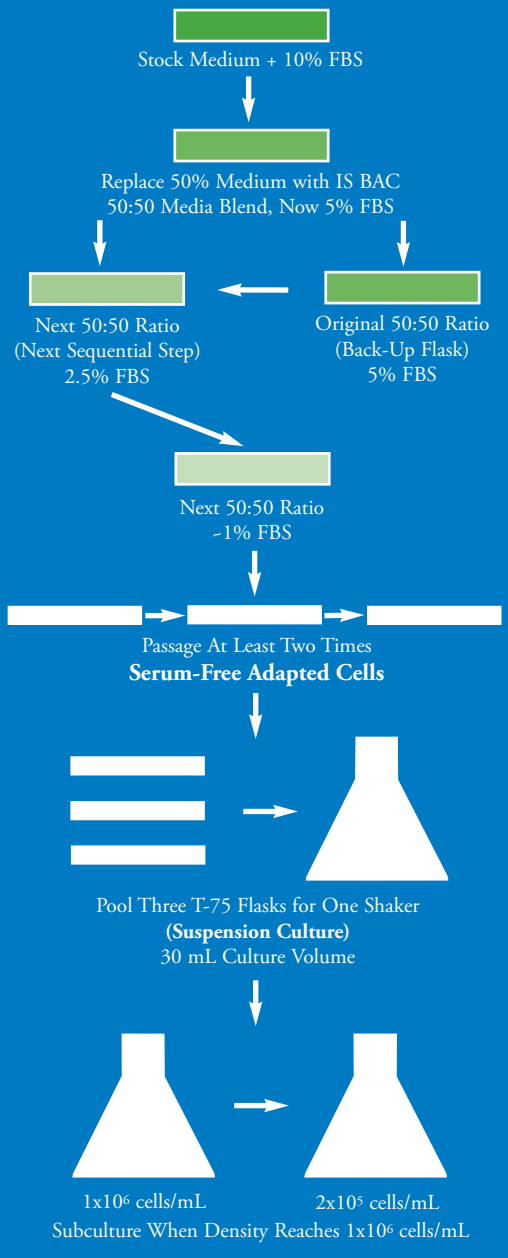
1. Cells can be grown as adherent monolayers and as non-adherent suspension cultures.
2. Cells grown in serum-supplemented (5%-10% FBS) or serum-free medium can be directly subcultured into serum-free IS BAC medium. It is critical that cells be in the mid-logarithmic phase of growth with at least 90% viability.
   A. Adherent monolayers can be subcultured directly into serum-free IS BAC on:
      1. Adherent culture (T-25, T-75, etc) at an initial density of 1:5 or
      2. Suspension culture (e.g., 125 mL shaker flasks with 30 mL culture volume and shaking at 160 rpm) at an initial density of $2x10^5$ cells/mL.
   B. Suspension cultures can be directly subcultured to $2x10^5$ cells/mL in serum-free IS BAC.
3. Incubate cultures accordingly at 37° C and 5% CO_2 until the viable cell density exceeds $2x10^6$ cells/mL with a viability greater than 90%.
4. Cells should be subcultured to $2x10^5$ cells/mL twice weekly when the viable cell density exceeds $2x10^6$ cells/mL, with viability greater than 90%.
5. 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 BAC. 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 BAC at an initial density of $2x10^5$ cells/mL in a 125 mL shaker flask (suspension culture).

Sequential Adaptation from Serum-Supplemented Media to Serum-Free IS BAC

1. Cells can be grown as adherent monolayers and as non-adherent suspension cultures.
2. Cells grown in 5%-10% FBS supplemented medium to a density of $1\text{-}3x10^6$ cells/mL should be subcultured to a density of $2x10^5$ cells/mL into a 50:50 ratio of serum-free IS BAC medium and the original serum supplemented medium.
3. Incubate accordingly (for adherent or suspension cultures) at 37° C and 5% CO_2 until the viable cell density exceeds $2x10^6$ cells/mL and viability is greater than 90%.
4. Subculture to a density of $2x10^5$ cells/mL into a 75:25 ratio of serum-free IS BAC medium and the original serum supplemented medium.
5. When the viable cell density exceeds $2x10^6$ cells/mL, with greater than 90% viability, subculture to $2x10^5$ cells/mL into 100% serum-free IS BAC medium. Higher seeding densities ($3\text{-}5x10^5$ cells/mL) may be required for the first few passages in IS BAC only. Once cells have adapted to growth in IS BAC alone, lower seeding densities can be used for subculturing.
6. Continue to subculture twice weekly when the viable cell density exceeds $2x10^6$ cells/mL. Continue passaging in IS BAC for several passages until the viable cell density exceeds $2x10^6$ cells/mL with greater than 90% viability. At this stage, the cells are adapted to IS BAC.
7. 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.

Sequential Adaptation Protocol



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

Irvine Scientific
2511 Daimler Street, Santa Ana, CA 92705

Customer Service: 1 800 577 6097 • Technical Service: 1 800 437 5706
• Telephone: 1 949 261 7800 • Fax: 1 949 261 6522

I.S. EN ISO 9001
I.S. EN 46001
REV.0