

BD Select™ CD1000 Medium

Adaptation Protocol

BD Select™ CD1000 is a chemically-defined, animal-free medium that is ideally suited for further hydrolysate or chemically-defined supplementation. This medium is formulated without L-Glutamine, Hypoxanthine, Thymidine, and Phenol Red. BD Select CD1000 is designed to support cell growth and protein production in CHO, NS0, and hybridoma cell lines.

Culture Recommendations

1. BD Select CD1000 should be supplemented with all necessary cell specific supplements (e.g GS Supplement, HT, L-Glutamine, or Cholesterol)
2. Cells should be seeded between $2.0 - 3.0 \times 10^5$ cells/mL
3. Cultures should be incubated at $37 \pm 2^\circ\text{C}$ and 5% CO_2

Direct Adaptation

Direct adaptation procedure can be used for the cells that may readily adapt into the new medium. Transfer cells growing in current medium directly into BD Select CD1000 at $3.0 - 5.0 \times 10^5$ cells/mL during the logarithmic growth phase. When viable cell density reaches $1.0 - 1.5 \times 10^6$ cells/mL, or the culture is in mid- to late-logarithmic growth phase, subculture the cells. Repeat the subculturing at least three times at 2-3 day intervals. If the viability drops below 80% during the procedure, adapt the cells sequentially as described below.

Sequential Adaptation

Complete adaptation from the old medium to BD Select CD1000 is achieved best in a four step, sequential process. First, seed the cells at $3.0 - 5.0 \times 10^5$ cells/mL in 25% BD Select CD1000 medium and 75% of the current culture medium. Cells should be seeded during the logarithmic growth phase with at least 80% (preferably greater than 90%) viability. Passage the cells in this media combination for **at least** 2-3 passages or until the cells retain normal doubling time and viability above 80%.

The next step is to further reduce the percentage of current culture medium by seeding in 50% of BD Select CD1000 medium and 50% of the current culture medium at $3.0 - 5.0 \times 10^5$ cells/mL. The cells should remain in this media combination for **at least** 2-3 passages, or until the cells retain normal doubling time and viability above 80%. (If the culture shows longer doubling time, more time should be given to adapt at this media combination).

In the next step of the process, seed the cells in 75% BD Select CD1000 medium and 25% of the current culture medium at $3.0 - 5.0 \times 10^5$ cells/mL. Allow **at least** 2-3 cell passages at this media combination, or until the cells retain normal doubling time and viability above 80% before moving the cells into the final step of the process.

Finally, seed cells at $3.0 - 5.0 \times 10^5$ cells/mL in 100% BD Select CD1000. Allow **at least** 3-4 passages in BD Select CD1000 before moving cells into the production system. It is best not to change to 100% BD Select CD1000 at a point when the cells can be passed as necessary. Once the cells are completely adapted (3-4 passages at 100% BD Select CD1000), a cell bank can be created. A freezing solution of 10% DMSO and 90% BD Select CD1000 is a recommended freezing solution.

Adaptation by Selection

Selection Adaptation process may be used when cells are unable to be weaned to BD Select CD1000 by either of the above mentioned processes.

1. Transfer cells growing in the current (original) medium directly into BD Select CD1000 at $3.0 - 5.0 \times 10^5$ cells/mL.
2. Allow cells to drop below 50% viability without subculturing.
3. After cells have fallen below 50% viability, centrifuge cells and re-suspend the entire pellet in the original medium at the appropriate culture volume.
4. Continue to culture cells in original medium until viability reaches $\geq 90\%$ for at least 3 passages. During this time, cells should be subcultured only after reaching a viable cell density $\geq 1.0 \times 10^6$ cells/mL or the culture is in mid- to late-logarithmic growth phase.
5. After cells have regained $\geq 90\%$ viability, passage the cells at **$4.0 - 5.0 \times 10^5$ cells/mL** in the appropriate culture volume back into BD Select CD1000.
6. Again, allow cells to drop below 50% viability without subculturing. Centrifuge cells and re-suspend entire pellet in original medium.
7. Repeat steps 4 through 6.
8. After 3-4 iterations of the above process, cells should transition to BD Select CD1000 on final cycle. Continue normal subculturing process.

For more information, including hydrolysate usage protocols, visit us today at bdbiosciences.com/advbio, call BD Technical Service & Support at **800.638.8663** or contact your Bioprocess Application Specialist.



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