gibco
by life technologies

CD BHK-21 Production Medium

Description

Gibco® CD BHK-21 Production Medium is a chemically defined, protein-free cell culture medium that does not contain protein hydrolysates or growth factors. This medium is designed for the growth of BHK-21 cells in serum-free suspension culture and it is suitable for large-scale manufacturing applications.

Product	Catalog No.	Amount	Storage	Shelf Life
CD BHK-21 Production Medium	A1627701 A1627702 A1627703	1 × 10 L 1 × 100 L 1 × 10 kg	Store at 2–8°C, dark and dry.	12 months from date of manufacture

Product Use

Caution: For manufacturing, processing, or repacking.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture Conditions

Media: CD BHK-21 Production Medium

Culture Type: BHK-21 cells in suspension

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5–8% CO_2 in air. Ensure that proper gas exchange is achieved in culture vessels. If CO_2 incubator is not available, use non-vented caps and ensure that flasks are tightly capped.

Prepare Culture Medium

1. Measure cool (15°C to 30°C) deionized or distilled water to 85% of final desired volume formulation vessel.
2. Slowly, add CD BHK-21 Production Medium (17.7 g/L)
3. Mix for 30 minutes.
4. Add sufficient sodium bicarbonate for final volume (2.32 g/L or 3.71 g/L)

   Note: The concentration of sodium bicarbonate in the medium must be matched to the level of CO_2 in the culture atmosphere. For use in 5–8% CO_2 culture conditions, the medium can be reconstituted with 2.32 g/L sodium bicarbonate. For CO_2-free culture conditions, we recommend 3.71 g/L sodium bicarbonate.
5. Mix for 10 minutes or until dissolved.
6. Adjust pH to a minimum of 7.25. Record pH.

   Note: The pH must be adjusted with 0.5N NaOH or HCl to bring it to a range of 7.25 ± 0.15. For applications such as virus production, the pH can be adjusted to 7.4 ± 0.10.
7. Dilute to final production volume with deionized or distilled water. Mix for 10 minutes.
8. Sterilize immediately by membrane filtration (positive pressure recommended).

Cell Adaptation

Start with a suspension BHK-21 cell line. Adaptation of suspension cells into a new medium typically requires higher seeding densities at early passages because a significant percentage of the culture may not survive the new environment. Seeding more cells in the culture will increase the number of viable cells for further passaging. We recommend attempting adaptation in small-scale culture (e.g., 125-mL shake flasks) to determine how quickly a BHK-21 cell line will take to the serum-free CD BHK-21 Production Medium. For best results, start with a culture with high viability (>90%). **Do not add serum to CD BHK-21 Production Medium.** Adaptation requires routine monitoring of cell density. You will find that cell viability may drop to very low levels. This is to be expected and it is an essential part of the adaptation process.

1. Start with a healthy BHK-21 cell culture in exponential growth in the current serum-containing medium.
2. Harvest cells from the serum-containing culture by gentle centrifugation at 200 × *g* for 10 minutes to remove all serum-containing medium.
3. Decant the serum containing medium and gently resuspend the cells in serum-free CD BHK-21 Production Medium at a density of 1×10^6 cells/mL. Do not pre-warm the medium.
4. Using 125-mL shake flasks with a total culture volume of 30 mL, incubate the culture at 36 to 38°C in a humidified atmosphere of 5–8% CO_2 in air on an orbital shaker platform rotating at 125 rpm. If seeding spinner flasks, adjust magnetic agitation to 40–60 rpm. If not using vented flasks, loosen flask caps to allow for gas exchange.

   Note: If CO_2 incubator is not available, use non-vented caps and ensure that flasks are tightly capped.
5. Monitor cell densities daily. Maintain cell density between 1×10^6 cells/mL and 2×10^6 cells/mL. If the cell density drops below the lower limit, wait 24 hours and count again. If the cell density is still below 1×10^6 cells/mL, centrifuge and resuspend the cells in the same medium to a density of 1×10^6 cells/mL. Ensure that culture volume is maintained between 20 mL and 30 mL.
6. If cell density goes above the upper limit of 2×10^6 cells/mL, adjust the volume by splitting the culture with fresh medium to 1×10^6 cells/mL. Continue monitoring the cultures daily.

Note: You may observe cell clumping during the adaptation process. We recommend gentle trituration by mixing the culture with a serological pipette:

i) Using aseptic technique, gently pipette up and down carefully to break the clumps.

ii) Swirl the culture clockwise and tilt the flask at a 45 degree angle to cause the clumps to settle, and then harvest the mostly single-cell suspension for counting.

iii) Perform the same process to harvest cells during passaging.

7. When the cells reach the point at which they require daily replacement of at least 50% medium volume, adaptation has been achieved.
8. Once the cell culture has been adapted to serum-free medium, it should be cultured over 5–10 passages by standard subculturing techniques. We recommend a seeding density of 0.3×10^6–0.5×10^6 viable cells /mL and subculturing every 48 hours.

BHK-21 Cryopreservation in Serum-free Medium

1. Culture desired quantity of cells in either shake or spinner flasks, harvesting in mid-log phase of growth with >90% viability.
2. Calculate the required volume of cryopreservation medium necessary for the number of vials. We recommend a freezing density of $>1 \times 10^7$ cells/mL.
3. On day of intended use, prepare the required volume of cryopreservation medium, consisting of fresh CD BHK-21 Production Medium and 7.5% DMSO. Store freezing medium at 4°C until use.
4. Pellet the cells from culture medium at 150 × *g* for 5–10 minutes. Gently sesuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2.0-mL cryovial).
6. Achieve cryopreservation in an automated or manual controlled rate freezing device following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase) storage at –125°C to –200°C.

   Note: Viability and recovery of cryopreserved cells should be checked 24 hours after storage of vials in liquid nitrogen.

Recovery

1. Add 30 mL of CD BHK-21 Production Medium to a 125-mL shake flask.
2. Rapidly thaw (<5 minute) a frozen vial in a 37°C water bath.
3. Aseptically transfer the entire contents of the cryovial into the shake flask and gently swirl shake flask to distribute the cells. Determine viability by Trypan Blue exclusion; culture density should be at least 3×10^5 viable cells/mL.
4. Incubate culture at 36 to 38°C in a humidified atmosphere of 5–8% CO_2 in air on an orbital shaker platform rotating at 125 rpm. If seeding spinner flasks, adjust magnetic agitation to 40–60 rpm. If not using vented flasks, loosen flask caps to allow for gas exchange.

   Note: If CO_2 incubator is not available, use non-vented caps and ensure that flasks are tightly capped.
5. Monitor cell growth at 24 hours. Subculture cells 1 to 2 days post thaw. It is recommended to subculture cells for a minimum of 3 passages before use in other applications.

Related Products

Product	Cat. No.
VP-SFM AGT™	12559
OptiPRO™ SFM (1X), Liquid	12309
Water, Distilled	15230

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Temperature Limitation	Manufacturer	Batch Code	Use By:	Catalog number
				
Caution, consult accompanying documents	Consult instructions for use	Protect from light	Sterilized using aseptic processing techniques	

Limited Product Warranty

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