Product Information

BHK-210 Pro (AD) Medium

Serum-Free, Chemically Defined Medium

BHK-210 Pro (AD) cell culture medium has been formulated for the growth of BHK-21 cells in serum-free suspension culture and is suitable for large scale manufacturing applications. BHK-210 Pro (AD) medium is a chemically defined (CD), serum-free (SF), animal origin-free (AOF) medium that contains no protein, hydrolysates, or components of unknown composition.

Application

BHK-210 Pro (AD) medium is shown to support high cell growth of BHK-21 suspension cell lines used for viral vaccine production. The medium is optimized in a way which does not necessitate complete medium exchange or cell sedimentation steps during the vaccine production process. This results in efficient viral infection process resulting in higher titers and can save precious time and power requirement. It is recommended to use only 50-70% of the maximum bioreactor volume for cell growth and the fresh medium of 30-50% may be added post achievement of desired cell density before viral infection process.

Specification Table

Classification	Serum	Animal Origin	Protein	Endotoxin	Antibiotics	Phenol Red
Chemically Defined	Free	Free	Free	< 5 EU/mL	No	Yes

Storage and Shelf-life

BHK-210 Pro (AD) medium is highly hygroscopic powder and should be stored in dry at 2°C to 8°C protected from light and moisture. The entire contents of each package should be used immediately after opening. Please refer to the label for the expiry date.

Culture conditions

Media: BHK-210 Pro (AD)
Cell line: BHK cells
Culture type: Suspension
Temperature range: 36°C to 38°C
Incubator atmosphere: Humidified atmosphere of 5-8% CO_2 in air**.**
Culture vessels: Shake flasks, spinner bottles, and bioreactor.

Recommendations

It is a prerequisite to adapt the BHK-21 cells to BHK-210 Pro (AD) medium in shaker bottles before scale-up to bioreactor. Please follow the adaptation instructions below.

Reconstitution of BHK Pro (AD) medium

1. Take 95 % of the final volume of cell culture grade water at an ambient temperature into an appropriately sized mixing vessel.
2. Add the dry powder medium (17.73 g/L) slowly to the water and rinse out the original package with a small amount of cell culture grade water to remove all traces of powder.
3. Allow to dissolve by gentle stirring (300 rpm) for 45-60 minutes.
4. Add 2.75 g of sodium bicarbonate per liter of the final volume of the medium and stir until dissolved (about 20 minutes).
5. Use 2N NaOH or 2N HCl to adjust the pH to 7.2-7.4 units, if required.
6. Add cell culture grade water to achieve the appropriate final volume and mix well.
7. Sterilize the medium immediately by using 0.22 µm filter.
8. Aliquot the sterile solution under aseptic conditions to avoid contamination, and store the medium at 2–8 °C, protected

from light.

Product Specification

Appearance	Off-white to creamish white; fine powder
Solubility	Soluble
Osmolality (without supplements)	240 – 290 mOsm/Kg
Osmolality (with supplements)	300 – 350 mOsm/Kg
pH (without supplements)	4.90 – 5.70
pH (with supplements)	6.90 – 7.40
Bacterial Endotoxin	≤ 5 EU/mL
Growth Promotion Assay	Complies

Adaptation of cells in BHK 210 Pro (AC) medium from serum containing classic medium

The proper growth of BHK-21 cells in BHK-210 Pro (AD) medium requires a careful adaptation to serum-free conditions. The cells need to be adapted to the BHK-210 Pro (AD) medium by sequential reduction of serum in the medium. Adaptation requires routine monitoring of cell health. It is possible that the cell viability may drop to very low levels during the process which is to be expected as cells take time to adapt to the new environment of serum-free conditions. The recommended adaptation procedure is mentioned below.

Adaptation Step 1

GMEM + 5% TPB + 10% FBS to BHK-210 Pro (AD) medium + 5% FBS

- Start with healthy BHK-21 cells (> 95% viability) having exponential growth in the current serum-containing medium (GMEM+TPB+FBS).
- Subculture cells from GMEM medium into BHK-210 Pro (AD) medium + 5% FBS at a concentration of 4 - 5 x 10^5 viable cells / mL in 250mL shaker bottles containing 100mL culture at 115-135 rpm, 5-8% CO_2 in air and 37 ^{0}C humidified atmosphere.
- Monitor cell growth every day. After 48 hours of inoculation or once the cell density is more than 2 x 10^6 cells / mL, whichever is later, passage the cells again using above procedure.
- Perform a minimum of 3 passages in this medium. Once the culture is stable with a viability of > 90%, proceed to the next step.
- Freeze some cells (WCB 5%, with 1 × 10^7 cells/mL in BHK-210 Pro (AD) medium, 5% FBS, 10% DMSO)

Adaptation Step 2

BHK-210 Pro (AD) medium + 5% FBS to BHK-210 Pro (AD) medium + 2% FBS

- Subculture cells from BHK-210 Pro (AD) medium + 5% FBS medium into BHK-210 Pro (AD) medium + 2% FBS at a concentration of 4 – 5 x 10^5 viable cells / mL in 250mL shaker bottles containing 100mL culture at 115-135 rpm, 5-8% CO_2 in air and 37 ^{0}C humidified atmosphere.
- Monitor cell growth every day. After 48 hours of inoculation or once the cell density is more than 2 x 10^6 cells / mL, whichever is later, passage the cells again using above procedure.
- Perform a minimum of 3 passages in this medium. Once the culture is stable with a viability of > 90%, proceed to the next step.
- Freeze some cells (WCB 2%, with 1 × 10^7 cells/mL in BHK-210 Pro (AD) medium, 2% FBS, 10% DMSO)

Adaptation Steps 3, 4 and 5

Repeat adaptation steps with step wise serum reduction

- Adaptation step 3 – from 2% FBS to 1% FBS
- Adaptation step 4 – from 1% FBS to 0.5% FBS
- Adaptation step 5 – from 0.5% FBS to 0% FBS (serum-free)

NOTE

- It is **not recommended to use antibiotics** during adaptation and regular passaging of cells in serum-free medium. If absolutely essential, the antibiotic concentration needs to be **5-10 folds lower** than what is used in serum-containing medium. Cells cultured in serum-free medium are susceptible to

antibiotics and hence should be avoided in such medium.

- Make a frozen stock of the cells in the original medium prior to adaptation.
- Keep a culture going of the cells in each prior condition when starting the next level of adaptation as a fall-back if the cells do not survive in the next passage.
- Make 2-3 vials during each step of sequential adaptation as a back-up.

Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth having viability >90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 1×10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of 90% BHK 210 Pro (AC) medium (with serum concentration depending on the stage of adaptation) + 10% DMSO and store at 4°C until use.
   NOTE: Prepare cryopreservation medium on the day of use.
4. Harvest cells by centrifugation at 100 × g for 5–10 minutes. Resuspend 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.
6. Achieve cryopreservation in an automated controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase) storage at –200°C to –125°C.
   Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen (see "Recover frozen cells" section).

Recover frozen cells

1. Rapidly thaw (about 1.5 minute) frozen cells in a 37°C water bath.
2. Transfer the contents of the cryovial into a 125-mL shake flask containing 25 mL BHK-210 Pro (AD) medium (with serum concentration depending on the stage of adaptation).
3. Incubate at 37°C in a humidified atmosphere of 5-8% CO2 in air with shaking speed of 115–135 rpm.
4. Monitor cell growth at 24 hours. Subculture cells 2 days post thaw. It is recommended to subculture cells for a minimum of 3 passages before use in other applications.

Subculture cells

1. Determine viable cell density using an automated cell counter or manual methods. Ensure that the cell density is ≥1.5×10^6 viable cells/mL, viability is ≥90%, and cells are in the mid-logarithmic phase prior to subculturing.
2. Calculate the volume of cell culture and medium necessary to seed a flask at $4 – 5 \times 10^5$ viable cells/mL in a total volume of 30 mL fresh BHK-210 Pro (AD) medium (with serum concentration depending on the different stages of adaptation) per 125-mL shake flask.
   Note: If cell density does not reach 1.5×10^6 viable cells/mL within 4 days of recovery, centrifuge cells at 100 × g for 5 minutes and resuspend the cell pellet in 25–30 mL of fresh BHK-210 Pro (AD) medium (with serum concentration depending on the different stages of adaptation). **DO NOT** reuse erlemeyer flask for subculturing as multiple subcultures in the same flask can lead to cell clumping.
3. Incubate at 37°C in a humidified atmosphere of 5-8% CO2 in air with shaking speed of 115–135 rpm.
4. Subculture cells by seeding at density of $4 - 5 \times 10^5$ viable cells/mL every 2 days with fresh BHK-210 Pro (AD) medium (with serum concentration depending on the different stages of adaptation).

Precautions and Disclaimer

This product is for research use only. Please consult the Material Safety Data Sheet for

information regarding hazards and safe handling practices. We provide information to our customers on application technologies and liability. Existing laws and regulations should be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information does not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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For any further questions regarding this product, please contact us at info@sarvoshadhi.com.

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