SAFC®
Pharma & Biopharma Raw Material Solutions

Product Information

Cellvento®
BHK-200 cell culture medium

with L-Glutamine and HEPES, without Sodium Bicarbonate and Phenol Red

Product Description

Cellvento® BHK-200 medium is a serum-free cell culture medium, formulated without any animal derived component and optimized for the culture of suspension BHK21 cells at high-density and viability and efficient propagation of viruses. BHK21 cells can be grown as suspension cultures in T-flasks, shaker flasks, spinner bottles or stirred tank bioreactors. Cellvento® BHK-200 medium has been developed for BHK-21 cell line but is not limited to this application and may suit other vaccine producing cell lines as well.

Application

Cellvento® BHK-200 medium has been shown to be an optimal formulation for the growth and maintenance of BHK21 suspension cell lines used for viral vaccine production, and qualified for instance for the production of Foot and Mouth Disease virus (FMDV).

When the density of BHK21 cells satisfies process requirement, the virus can be inoculated directly to the reactor without media exchange or cell separation/ sedimentation steps, resulting in highly efficient virus production. It is recommended to use only 70% of the maximum bioreactor volume for cell growth. After cells have reached the desired cell density, 30% fresh medium can be added in the reactor before inoculation and propagation of the virus.

Recommendations

It is an important prerequisite to adapt the BHK21 cells to the serum-free conditions in shaker bottles before scale-up to bioreactor volumes. Please follow the adaptation instructions below. For additional advice and assistance please contact our technical service department through vaccinemedia@merckgroup.com.

Precautions

Powdered media are hygroscopic and should be protected from moisture. The entire content of each package should be used immediately after opening. Preparing a concentrated solution of medium is not recommended as precipitates may form.

Media preparation instructions for 10 L

For the media preparation, sodium bicarbonate needs to be added (Art. No. 1.37013) following the instruction below.

1. Measure out 9.5 L of cell culture grade (Milli-Q®) water, by weight, into a 10 L carboy or equivalent.
2. Slowly add 216.655 g of Cellvento® BHK-200 medium powder to the water.
3. Allow to dissolve by gentle stirring (300 rpm) for 15 minutes.
4. Add sodium bicarbonate (20 g/10 L) and stir until dissolved (~15 minutes).
5. Measure the pH (should be approximately 6.85) and adjust the medium using 2M sodium hydroxide (approx. 14-16 mL /10 L) to a pH of 7.1-7.2.
6. Fill up to 10 L final volume using Milli-Q® water.
7. Sterilize by membrane filtration with Millipore Express® Plus membrane (0.22 µm, Polyethersulfone).
8. Measure the final pH and osmolality (pH = 7.2-7.4; osmolality = 362 +/-15 mosmol/kg).
9. Store at 2–8 °C. Protect from light.

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

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Storage

Store the dry powder medium at 2-8 °C under dry conditions, and the filtered liquid medium at 2-8 °C protected from light. Do not use after the expiration date.

Specifications

Item	Description
Appearance	off-white to light brown; fine powder
Solubility	well soluble
pH (without supplements)	5.7 - 6.3
pH (with supplements)*	6.5 - 7.1
Osmolality (without supplements):	293-333 mosmol/kg
Osmolality (with supplements)*:	335-375 mosmol/kg
Bacterial endotoxins (EU/mL):	≤ 10
Cell growth test (BHK21)**	After 72 hours cultivation, cell quantity is more than 1.5 × 10^6 cells/mL, cell viability is more than 90%

* pH and osmolality with supplements refer to the values obtained after bicarb supplementation. Final values for the ready-to-use liquid medium are pH 7.2-7.4 and osmolality 362 +/-15 mosmol/kg.

** Cell growth test is assessed by using BHK21 suspension cell line. Cells are seeded at a density of 3 × 10^5 cells/mL and cultivated for 72 hours in shaker flasks in standardized cell culture conditions.

Ordering information

Cat. No.	Product Name	Pkg. size	Equivalent
1.02376.0010	Cellvento® BHK-200 medium	216.655 g	10 L
1.02376.0100	Cellvento® BHK-200 medium	2.167 kg	100 L
1.02376.1000	Cellvento® BHK-200 medium	21.67 kg	1000 L

Adaptation of cells to serum-free growing conditions in Cellvento® BHK-200 cell culture medium

The successful growing of BHK21 cells in Cellvento® BHK-200 medium requires a careful adaptation to serum-free conditions. Following the detailed procedure below, BHK21 cells need to be adapted via a progressive reduction of the percentage of serum.

1st Adaptation step (GMEM + 5% TPB + 10% FBS → Cellvento® BHK-200 medium + 10% FBS)

- Start with BHK21 cells cultured in suspension in their current medium, usually GMEM+10% FBS (Fetal Bovine Serum or NCS, Newborn Calf Serum) + 5% TPB (Tryptose Phosphate Broth) in shaker flask at a cell density of 5 × 10^6 cells/mL with > 95% viability.
- Passage from the GMEM medium to Cellvento® BHK-200 medium + 10% FBS. Subculture at a concentration of 6 × 10^5 cells/mL.
- Perform minimum 3 passages (100 mL in 250 mL shaker bottles at 110 rpm). Passage when VCD (viable cell density) is above 1.5 × 10^6 cells/mL
- When the culture is stable with a viability of > 95% and a cell density above 1.5 × 10^6 cells/mL, continue with the next step additional 2 passages in 250 mL shaker flasks.

2nd Adaptation step (Cellvento® BHK-200 cell culture medium + 10% FBS → Cellvento® BHK-200 medium + 7% FBS)

- Passage from Cellvento® BHK-200 medium + 10% FBS to Cellvento® BHK-200 medium + 7% FBS. Subculture at a concentration of 6 × 10^5 cells/mL.
- Perform minimum 3 passages (100 mL in 250 mL shaker bottles at 110 rpm). Passage when VCD is above 1.5 × 10^6 cells/mL
- When the culture is stable with a viability of > 95% and a cell density above 1.5 × 10^6 cells/mL
- Freeze some cells (WCB 7%, with 1 × 10^7 cells/mL in Cellvento® BHK-200 medium, 7% FBS, 10% DMSO)

Repeat adaptation steps with a progressive reduction of serum

- 3rd Adaptation step from 7% to 5%
- 4th Adaptation step from 5% to 3%
- 5th Adaptation step from 3% to 1%
- 6th Adaptation step from 1% to 0.5%
- 7th Adaptation step from 0.5% to 0%

8th Adaptation step (low density culture in Cellvento® BHK-200 cell culture medium)

- Passage in Cellvento® BHK-200 medium. Subculture at a concentration of 3×10^5 cells/mL.
- Perform minimum 3 passages (100 mL in 250 mL shaker bottles at 110 rpm). Passage when VCD is above 1.5×10^6 cells/mL
- When the culture is stable with a viability of > 95% and a cell density above 1.5×10^6 cells/mL.
- Prepare a MCB (min. 30 tubes in Cellvento® BHK-200 medium, 10% DMSO, 1×10^7 cells/mL)
- Prepre a WCB (min. 100 tubes in Cellvento® BHK-200 medium, 10% DMSO, 1×10^7 cells/mL)

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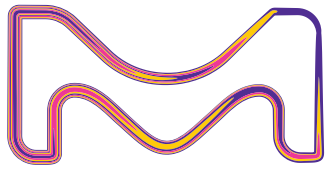

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