

Product Information

1.02376 Cellvento™ BHK-200 Cell culture medium

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

Product description

Cellvento™ BHK-200 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.

Application

Cellvento™ BHK-200 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 vaccinemia@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 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 (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.

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.

Shelf life


12 months for the dry powder medium.

Specification

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):	285 - 315 mosmol/kg
Osmolality (with supplements)*:	326 - 361 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 × 10⁵ cells/mL and cultivated for 72 hours in shaker flasks in standardized cell culture conditions.

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Ordering information

Catalog number	Product name	Package size	Equivalent
1.02376.0010	Cellvento™ BHK-200	216.655 g	10 L
1.02376.0100	Cellvento™ BHK-200	2.167 kg	100 L
1.02376.1000	Cellvento™ BHK-200	21.67 kg	1000 L

Adaptation of cells to serum-free growing conditions in Cellvento™ BHK-200

The successful growing of BHK21 cells in Cellvento™ BHK-200 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 + 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 + 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

2nd Adaptation step (Cellvento™ BHK-200 + 10% FBS → Cellvento™ BHK-200 + 7% FBS)

- Passage from Cellvento™ BHK-200 + 10% FBS to Cellvento™ BHK-200 + 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, 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)

- Passage in Cellvento™ BHK-200. 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, 10% DMSO, 1×10^7 cells/mL)
- Prepare a WCB (min. 100 tubes in Cellvento™ BHK-200, 10% DMSO, 1×10^7 cells/mL)

For more information and documentation please contact:

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The typical technical data above serve to generally characterize the cell culture media in industry-relevant expression systems. The product information is available separately from the website www.merckmillipore.com

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