Instructions for Use

4Cell® BHK-21 CD Medium

2582843-000-03

SARTORIUS

Contents

1. Introduction

4Cell® BHK-21 CD Medium is a chemically defined, serum-free, protein-free, and animal-component free medium formulated to maximize the production of vaccine in BHK-21 (Baby Hamster Kidney) suspension cells with proven robust performance in large-scale manufacturing. 4Cell® BHK-21 CD Medium does not require serum supplementation.

1.1 Intended Use and Safety Statements

4Cell® BHK-21 CD Medium is **for Research or Further Manufacturing Use**.
Not approved for human or veterinary use. Not for application in humans or animals, or for use in *in vitro* diagnostic or clinical procedures.
Please follow the handling instructions in the Material Safety Data Sheets (MSDSs).

1.2 Stability

- 4Cell® BHK-21 CD Medium liquid formulation already contains L-Glutamine.
- 4Cell® BHK-21 CD Medium powder formulation contains L-Glutamine.

1.3 Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. When not in use store 4Cell® BHK-21 CD Medium at 2°C to 8°C protected from light.

1.4 Suggested Materials

- BHK-21 suspension cells
- 4Cell® BHK-21 CD Medium (see Order No.)
- Erlenmeyer cell culture flask, ambr® 15 | 250, Biostat® and Biostat STR® bioreactors
- 100 – 400 g/L sterile filtered Glucose
- PES membrane filter with 0.2 µm pore size, e.g. Sartopore® 2

2. Instructions for Use

All procedures should be carried out in a Biological Safety Cabinet under sterile conditions. Before starting the experiments examine the cells under the microscope to ensure they are healthy and free of contamination.
Always pre-warm the medium to 37°C prior to use and follow the cell cultivation parameters below.

2.1 4Cell® BHK-21 CD Medium Powder Formulation Reconstitution (Optional)

1. Fill deionized or distilled water (at room temperature 15 - 25°C) into the mixing vessel. To allow pH adjustment later on, the volume should be 95 % of the final volume.
2. Add 22.64 g/L of 4Cell® BHK-21 CD Medium dry powder and stir for 30 minutes.
3. Add 2.20 g/L of sodium bicarbonate ($NaHCO_3$) and mix for 15 minutes or until completely dissolved.
4. Measure the pH.
5. Adjust the pH of the 4Cell® BHK-21 CD Medium to 7.0 – 7.3 or at the desired range.
6. Fill with water to the final volume and mix to combine.
7. Measure and record the final pH and osmolality of the 4Cell® BHK-21 CD Medium. Expected traits:
   - pH target: 7.10 ± 0.10 (for virus production, the pH range can be 7.3 ± 0.10)
   - Osmolality target: 310 ±10 mOsmol/kg
   - Color: transparent yellow to faint red
8. Sterilize the medium using a PES membrane filter with 0.1 µm pore size (Sartopore® 2).
9. Store at 2 - 8°C. Protect from light.

2.2 Adapting BHK-21 cell lines to 4Cell® BHK-21 CD Medium

2.3.1 Adapting suspension BHK-21 cells growing in serum-free conditions

BHK-21 suspension cells can be directly adapted from serum-free medium to 4Cell® BHK-21 CD Medium (Option 1).
Some BHK-21 cells will require sequential adaptation (Option 2).

- **Option 1 Direct adaptation:** Passage the culture directly from the initial (reference) medium into 4Cell® BHK-21 CD Medium. For direct adaptation, the cell inoculum should be 5×10^5 viable cells/mL.

1. Subculture the BHK-21 suspension cells in your reference serum-free medium for at least 3 passages before using 4Cell® BHK-21 CD Medium.

Note
See cell cultivation for incubation parameters.

2. When the cells achieve a stable growth rate and viability >90%, inoculate the culture vessel with 5×10^5 viable cells/mL in 4Cell® BHK-21 CD Medium.
3. Continue this sub-cultivation in 4Cell® BHK-21 CD Medium for at least 4 passages or until stable doubling times are obtained and viability >90%.
4. Stock cultures of BHK-21 suspension cells adapted to 4Cell® BHK-21 CD Medium should be subcultured in 4Cell® BHK-21 CD Medium every 2 to 3 days when the cell density is 2×10^6 to 4×10^6 cells/mL with 90% viability.
5. Proceed with a freezing step (see freezing of Cells | Storage).

- **Option 2 Sequential adaptation:** Passage the culture into a mixture of reference culture medium and 4Cell® BHK-21 CD Medium and gradually increase the content of 4Cell® BHK-21 CD Medium. For sequential adaptation, the cell inoculum should be 1×10^6 viable cells/mL.
  An example for a stepwise BHK-21 cells adaptation protocol is given below.

Adaptation step	Ratio of serum-free reference medium to 4Cell® BHK-21 CD Medium	Acceptance criterion to proceed to next adaptation step
1	75:25	Viability ≥90% of reference medium; doubling time of ≤48h and stable growth for 3 passages
2	50:50	Viability ≥90% of reference medium; doubling time of ≤48h and stable growth for 3 passages
3	25:75	Viability ≥90% of reference medium; doubling time of ≤48h and stable growth for 3 passages
4	0:100	Adaptation complete if viability >90% in 4Cell® BHK-21 CD Medium; specific growth rate range: $\mu > 0.028h^{-1}$ and $0.032h^{-1}$ constant cell growth rate for 3 passages

2.3.2 Adapting adherent BHK-21 cells growing in serum containing conditions

To adapt anchorage-, serum-dependent BHK-21 cells growing in adherent conditions to suspension serum-free conditions using 4Cell® BHK-21 CD Medium please refer to **Application note: Adaptation of BHK-21 cells to suspension using 4Cell® BHK-21 CD Medium.**

2.3 Cell Cultivation

- Cultivate the cells at at 36.5°C ± 0.5°C in 80% humidity and in an atmosphere of 5-7% CO_2 with constant (rotational) speed of 100 rpm or equivalent.
- Other cultivation parameters may be adapted to each BHK-21 cell line's individual requirements.
- By regular passaging of the cells, ensure that the culture remains in mid-exponential growth phase at all times. Determine cell density and viability of the culture every 2 - 3 days and dilute the culture to a suitable seeding density (3×10^5 - 5×10^5 viable cells/mL) with fresh pre-warmed 4Cell® BHK-21 CD Medium.

Note
Supplement cultures with additional glucose (up to 7 g/L), when glucose concentrations are below 4 g/L to prevent depletion.
Add L-glutamine between 4-8mM if required.

2.4 Thawing of Cells | Initiation of Culture Process

The required 4Cell® BHK-21 CD Medium volume depends on the cell density in frozen cryovials. The cell density after thawing should be 4 - 5×10^5 viable cells/mL.

1. After removing cryovial from storage, wipe the cryovial with 70% v/v ethanol or isopropanol before opening. In a Biological Safety Cabinet, briefly twist the cap a quarter turn to relieve pressure, and then retighten.
2. Quickly thaw the cryovial in a 37°C water bath (do not submerge the cryovial completely) or heating block at 37°C until only a small grain of ice remains. Thawing the cells for longer than 2 minutes may result in reduced cell viability.
3. Dry the cryovial with a lint-free wipe, spray with 70% v/v ethanol or isopropanol, and then wipe to remove excess liquid.
4. Gently add the thawed cell suspension to a sterile conical tube containing at least 10 mL of pre-warmed 4Cell® BHK-21 CD Medium. Centrifuge at 200 × *g* for 10 minutes at room temperature.
5. Remove the supernatant carefully and reconstitute the cell pellet with 1 mL in fresh pre-warmed 4Cell® BHK-21 CD Medium. Mix by pipetting up and down the suspension.
6. Count the cells and transfer the suspension as inoculum into the culture vessel containing fresh pre-warmed 4Cell® BHK-21 CD Medium. Proceed with cell cultivation as described above.

2.5 Freezing of Cells | Storage

The cell culture should be in mid-logarithmic growth phase and >90% viable at the point of freezing.

1. Prepare the necessary volume of freezing medium by supplementing 4Cell® BHK-21 CD Medium with 10% Dimethylsulfoxide (DMSO). Store the freezing medium at 2 – 8°C until use.
2. Transfer the required volume of cell suspension into centrifugation vessels and spin down the cells at 180 – 200 × g for 10 minutes. Gently remove the supernatant.
3. Reconstitute the cell pellet in the required volume of freezing medium that has been cooled to 2 - 8°C to achieve a cell density of 1-2 × 10^7 viable cells/mL.
4. Transfer the cell suspension to each sterile cryovials, taking care that the suspension remains homogenous.
5. Place the vials in a control rate freezer or pre-cooled (2 - 8°C) freezing container overnight until -80°C.
6. Transfer and store the cryovials for long-term storage at a temperature below -130°C.

Note
Check viability and recovery of BHK-21 cryopreserved cells 48 hours after storage in liquid nitrogen.

3. References | Contacts | Order No.

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Product*	Size & Package*	Material No.
4Cell® BHK-21 CD Medium (Powder)	10 L | 1 Bucket	CQV3FA0010
4Cell® BHK-21 CD Medium (Powder)	50 L | 1 Bucket	CQV3FA0011
4Cell® BHK-21 CD Medium (Powder)	100 L | 1 Bucket	CQV3FA0014
4Cell® BHK-21 CD Medium (Liquid)	500 mL | 2 x 500 mL Bottle	CFV3FA0002
4Cell® BHK-21 CD Medium (Liquid)	500 mL | 6 x 500 mL Bottle	CFV3FA0001

* Other sizes and formats are available on request

The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice. Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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