Instructions for Use

4Cell® MDCK CD Medium

2582845-000-04

SARTORIUS

Contents

1. Introduction

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

1.1 Intended Use and Safety Statements

4Cell® MDCK 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 (MSDS).
4Cell® MDCK CD Medium is ready to use and does not require additional supplementation of external components.

1.2 Unpacking and Storage Instructions

1. Check all containers for leaks or damage.
2. When not in use store 4Cell® MDCK CD Medium at 2°C to 8°C protected from light.

1.3 Suggested Materials

- MDCK suspension cells
- 4Cell® MDCK CD Medium (see Order No.)
- Erlenmeyer cell culture flask, ambr® 15 | 250, Biostat® and Biostat STR® bioreactors
- 100 – 400 g/L sterile filtered Glucose
- L-Glutamine, stock solution 200 mM if required
- 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.

1.4 4Cell® MDCK 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, the volume should be 95 % of the final volume.
2. Add 22.21 g/L of 4Cell® MDCK CD Medium dry powder and stir for 30 minutes.
3. Add 0.25 g/L of sodium hydroxide in powder form, to adjust the pH to approximately 6.2 - 6.7, and mix for 15 minutes.
4. Add 2.00 g/L of sodium bicarbonate ($NaHCO_3$) and mix for 15 minutes or until completely dissolved.
5. Measure the pH.
6. Adjust the pH of the 4Cell® MDCK CD Medium with 1M HCL solution to 7.0 – 7.3 or at the desired range.
7. Fill with water to the final volume and mix to combine.
8. Measure and record the final pH and osmolality of the 4Cell® MDCK CD medium. Expected traits:
   - pH target: 7.15 ± 0.15
   - Osmolality target: 300 ±15 mOsmol/kg
   - Color: transparent yellow to faint red
9. Sterilize the medium using a PES membrane filter with 0.2 µm (or 0.1 um) pore size (Sartopore® 2).
10. Store at 2 - 8°C. Protect from light.

1.5 Adapting MDCK cell lines to 4Cell® MDCK CD Medium

2.2.1 Adapting suspension MDCK cells growing in serum-free conditions
MDCK suspension cells can be directly adapted from serum-free medium to 4Cell® MDCK CD Medium (Option 1). Some MDCK cells will require sequential adaptation (Option 2).

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

1. Subculture the MDCK suspension cells in your original serum-free medium for at least 3 passages before using 4Cell® MDCK 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® MDCK CD Medium.
3. Continue this sub-cultivation in 4Cell® MDCK CD Medium for at least 4 passages or until stable doubling times (~ 30 h) are obtained and viability >90%.
4. Stock cultures of MDCK suspension cells adapted to 4Cell® MDCK CD Medium should be subcultured in 4Cell® MDCK 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 section 2.5, Freezing of Cells | Storage).

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

Adaptation step	Ratio of serum-free reference medium to 4Cell® MDCK CD Medium	Acceptance criterion to proceed to next adaptation step
1	75:25	Viability ≥90% of reference medium; doubling time of ≤35h and stable growth for 2-3 passages
2	50:50	Viability ≥90% of reference medium; doubling time of ≤35h and stable growth for 2-3 passages
3	25:75	Viability ≥90% of reference medium; doubling time of ≤35h and stable growth for 2-3 passages
4	10:90	Viability ≥90% of reference medium; doubling time of ≤35h and stable growth for 2-3 passages
5	1:99	Viability ≥90% of reference medium; doubling time of ≤35h and stable growth for 2-3 passages
6	0:100	Adaptation complete if viability >90% in 4Cell® MDCK CD Medium; specific growth rate range: $\mu > 0.028h^{-1}$ and $0.033h^{-1}$ constant cell growth rate for 2-3 passages

2.2.2 Adapting adherent MDCK cells growing in serum containing conditions

To adapt anchorage, serum dependent MDCK cells growing in adherent Conditions to suspension serum-free conditions, passage the culture into a mixture of serum-required medium and 4Cell® MDCK CD Medium and gradually increase the content of 4Cell® MDCK CD Medium. For sequential adaptation, the cell inoculum should be 1.0×10^6 viable cells/mL.
An example for a stepwise MDCK cells adaptation protocol is given below.

1. Grow enough adherent MDCK cells using your reference serum-containing medium and maintain within log phase (cells should have < 80% confluency).
2. Take a sample of MDCK cells to determine cell density and viability (recommended viable cell density > 1.0×10^6 cells/mL, viability > 90%).
3. Inoculate $1.0 - 1.5 \times 10^6$ cells/mL into the culture vessels containing 25% of 4Cell® MDCK CD Medium and 75% of the reference serum-containing medium.
4. Incubate at 37°C in a 5% CO_2 environment with constant (rotational) speed of 100 rpm or equivalent.
5. After 48h, if cell density doubles and viability ≥ 90%, passage the cells with the next cell culture media rate mixture (50%; than 75%, 90%, 99% and finally 100%). If density and viability is not achieved, the cells should be maintained (and passaged) with current adaption medium until stable cell growth can be obtained.
6. Repeat Step 5 until concentration of 100% serum-free medium (4Cell® MDCK CD Medium) is achieved.
7. The adaptation is considered complete if viability ≥ 90% in 4Cell® MDCK CD Medium; specific growth rate: $\mu > 0.33\ d^{-1}$ and constant cell growth rate for 3 passages.

1.6 Cell Cultivation

- Cultivate the cells at 36.5°C ± 0.5°C in an atmosphere of 5% CO_2 with constant (rotational) speed of 100 rpm or equivalent.
- Other cultivation parameters may be adapted to each MDCK 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 (4×10^5 - 5×10^5 viable cells/mL) with fresh pre-warmed 4Cell® MDCK CD Medium.

Important
Depending on cell line and cultivation conditions glucose and L-Glutamine may be supplemented.
- **Supplement cultures with additional glucose (up to 7 g/L), when glucose concentrations are below 3 g/L to prevent depletion.**
- **Supplement cultures with additional L-Glutamine (up to 8 mM), when L-Glutamine concentrations are below 3 mM to prevent depletion.**

1.7 Thawing of Cells | Initiation of Culture Process

The required 4Cell® MDCK CD Medium volume depends on the cell density in frozen cryovials.

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 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® MDCK 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 of pre-warmed 4Cell® MDCK 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® MDCK CD Medium. Proceed with cell cultivation as described above.

1.8 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® MDCK CD Medium with 10% Dimethylsulfoxide (DMSO). Store the freezing medium at 2 – 8°C until use.
2. Centrifuge the required amount of cell suspension at 200 × *g* for 10 minutes at room temperature. Gently aspirate 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 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 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 MDCK cryopreserved cells 48 hours after storage in liquid nitrogen.

3. References | Contacts | Order No

Please refer to your respective sales contact.

Product*	Material No.
4Cell® MDCK CD Medium 2x1L Bottles	CFV3FA2003
4Cell® MDCK CD Medium 6x1L Bottles	CFV3FA2004
4Cell® MDCK CD Medium 10L Powder	CQV3FA2012
4Cell® Media CD Medium 100L Powder	CQV3FA2014

*Other sizes 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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