Instructions for Use

# 4Cell® Insect CD Medium


2582844-000-02

SARTORIUS

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# 1. Introduction

4Cell® Insect CD Medium is a chemically defined, serum-free, protein-free, animal-component free medium formulated to maximize the production of baculovirus from Sf9 (Spodoptera frugiperda) cells. It provides robust performance in production systems of small and large scale, e.g. flask, Ambr® 15|250 and bioreactors.

## 1.1 Intended Use and Safety Statements

4Cell® Insect 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® Insect CD Medium liquid formulation already contains L-Glutamine and do not require L-Glutamine supplementation prior to use.
- During the cultivation of Sf9 cells, it may be necessary to supplement cultures with additional L-Glutamine, usually at 6 – 8 mM final concentration, to prevent L–Glutamine depletion.

## 1.3 Unpacking and Storage Instructions

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

## 1.4 Suggested Materials

- *Spodoptera frugiperda* cells
- 4Cell® Insect 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
- 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 room temperature (RT, 20°C to 25°C) protected from light prior to use and follow the incubation parameters below.

## 2.1 Adapting insect cell lines to 4Cell® Insect CD Medium

### 2.1.1 Adapting Sf9 cell lines growing in serum-free conditions

Sf9 cells can be directly adapted from serum-free medium to 4Cell® Insect CD Medium (Option 1). For other insect cells sequential adaptation is needed (Option 2).

- **Option 1 Direct adaptation:** Passage the culture directly from the initial (reference) medium into 4Cell® Insect CD Medium. For direct adaptation, the cell inoculum should be 5 – 8 × $10^5$ viable cells/mL.

1. Subculture the Sf9 cells when the cell density is 2 × $10^6$ cells/mL to 3 x $10^6$ cells/mL. Note: see cell cultivation for incubation parameters.
2. When the cells achieve a stable growth rate and viability >90% (normally after 10 passages consecutively), the adaptation is considered complete.
3. Stock cultures of Sf9 cells adapted to 4Cell® Insect CD Medium should be subcultured in 4Cell® Insect CD Medium every 3 to 4 days at 5 × $10^5$ cells/mL.
4. 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® Insect CD Medium and gradually increase the content of 4Cell® Insect CD Medium. . For sequential adaptation, the cell inoculum should be 1 × $10^6$ viable cells/mL. An example for a stepwise insect cells adaptation protocol is given on the next page.

| Adaptation step | Ratio of serum-free reference medium to 4Cell® Insect 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 | 12:88 | Viability ≥90% of reference medium; doubling time of ≤48h and stable growth for 3 passages |
| 5 | 5:95 | 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® Insect CD Medium; specific growth rate range: $\mu > 0.024h^{-1}$ and $0.028h^{-1}$ constant cell growth rate for 3 passages |

## 2.2 Cell Cultivation

- Cultivate the cells at 27°C ± 1°C with constant (rotational) speed of 100-120 rpm.
- Other cultivation parameters may be adapted to each insect 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 ($4 \times 10^5$ - $5 \times 10^5$ viable cells/mL).

**Important**

- **After 96 hours of culture, sample each culture for glucose and L-Glutamine.**
- **Supplement cultures with additional glucose (up to 7 g/L), when glucose concentrations are below 4 g/L to prevent depletion.**
- **Supplement cultures with additional L-Glutamine (up to 8 mM), when L-Glutamine concentrations are below 4 mM to prevent depletion.**

## 2.3 Thawing of Cells | Initiation of Culture Process

The required 4Cell® Insect CD Medium volume depends on the cell density in frozen cryovials. The cell density after thawing should be 6 - $8 \times 10^5$ viable cells/mL.

1. 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 3 minutes may result in reduced cell viability.
2. Immediately transfer the thawed cell suspension with a pipette into 10 mL of RT 4Cell® Insect CD Medium. Centrifuge at 300 × *g* for 10 minutes at room temperature.
3. Remove the supernatant carefully and reconstitute the cell pellet with 2 mL in fresh RT 4Cell® Insect CD Medium. Mix by pipetting up and down the suspension.
4. Count the cells and transfer the suspension as inoculum into the culture vessel containing fresh RT 4Cell® Insect CD Medium. Proceed with cell cultivation as described above.

## 2.4 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® Insect 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 300 × *g* for 10 minutes, preferably at 4°C. Gently remove the supernatant.
3. Reconstitute the cell pellet in the required volume of freezing medium to achieve a cell density of at least $2 \times 10^7$ viable cells/mL. Dispense the suspension into cryovials, taking care that the suspension remains homogenous at all times.
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 Sf9 cryopreserved cells 48 hours after storage in liquid nitrogen.

**Note**
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## 2.5 Viral Infection of Sf9 cells with Baculovirus expression system

Follow the procedure Initiation of Culture Process to prepare the cells for infection. Viral infections should be carried out at cell densities between 2 – 4 x $10^6$ viable cells / mL under a Biological Safety Cabinet with all necessary safety precautions taken. See a protocol example below.
Proceed with infection only when the cells are fully adapted to the medium (See paragraph on adaptation above).

**Note**
**Viral Infection serve as a guideline and different cell densities, multiplicity and time of infection may be needed for your insect cells and should be adjusted accordingly. Avoid prolonged exposure to temperature higher than 29°C as it will cause cell death.**

The following protocol in best carried out in ambr® 15 | 250, Biostat® and Biostat STR® bioreactors, alternatively Erlenmeyer flask can be used.

1. Determine cell density and viability of the culture.
2. Determine the amount of virus required for the infection. The optimum multiplicity of infection (MOI) for baculovirus amplification is MOI = 0.1 and higher MOI is preferred for protein expression (MOI = 2).
3. Prepare infection mix: If volume of virus stock is < than 1 mL then dilute with 4Cell® Insect CD medium to 1 mL final volume. If volume of virus stock is greater than 1 mL go to next step.
4. Infect the cells by adding the infection mix to the container containing the cells.
5. Incubate the infected cells at 27°C ± 1°C, for 20 to 30 min with constant (rotational) speed of 100-120 rpm.
6. Harvest the culture after 1-4 days (usually 3 days, 72h) post-transfection by transferring the cell culture into 50 mL conical tubes.
7. Centrifuge the cell suspension at 200 x *g* for 10 minutes at room temperature. Collect and filter the virus-containing supernatant using a 0.2 µm PES filter unit. The filtered supernatant can be stored at 4°C for 3-4 days or at -80°C with sucrose for longer period of time.

**Note**
**Alternatively if the protein of interest is not secreted and remains intracellular, centrifuge the cell suspension at 1000 x *g* for 5 minutes, discard the supernatant and store the pellet at -80°C prior to protein purification.**

# 3. References | Contacts | Order No.

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| Product* | Material No. |
|---|---|
| 4Cell® Insect CD Medium 2x1 L Bottles | CFV3FA10001 |
| 4Cell® Insect CD Medium 6x1 L Bottles | CFV3FA10002 |

*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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