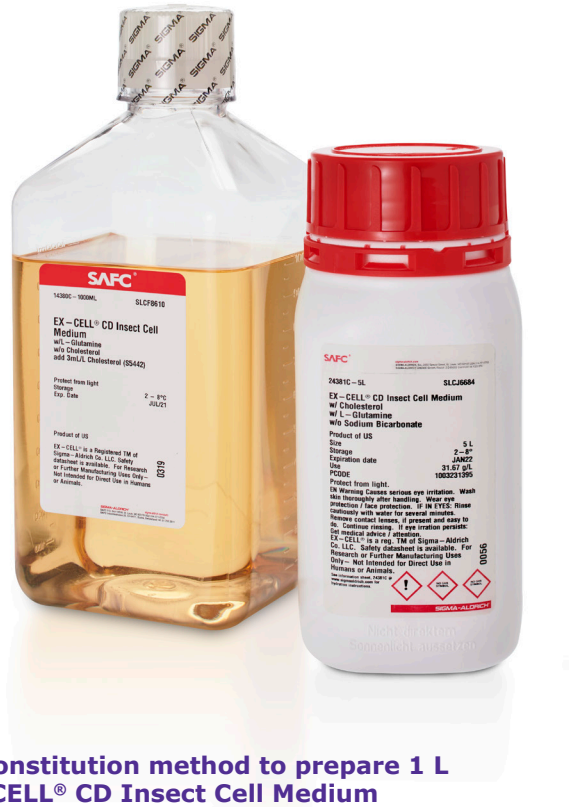
SAFC®
Pharma & Biopharma Raw Material Solutions

Product Information Sheet

EX-CELL® CD Insect Cell Medium

A chemically defined medium specially formulated to get the best performances for insect cell lines



Product Description

EX-CELL® CD Insect Cell Medium is a chemically defined cell culture medium designed specifically to get best performances for the insect cell lines. The formulation is animal-component free and has been formulated with L-Glutamine.

Application

EX-CELL® CD Insect Cell Medium can be used as amplification and production medium for *Spodoptera frugiperda* (Sf) cells such as Sf21 and Sf9 cells and is compatible with Tni, C636 and S2 cells. The media has been optimized to get excellent growth and productivity for the rhabdoviruse-free Sf-RVN® Insect Cell Line (Sf9). Together those products form the Sf-RVN® Platform.

This product is intended for research or further manufacturing but not for human or therapeutic use.

Media preparation:

Liquid medium must be supplemented with 3 mL/L of SyntheChol®.

Dry powder medium must be supplemented with 0.760 g/L Sodium Bicarbonate. No SyntheChol® supplementation is required.

Storage

Liquid and dry powdered media should be stored at 2-8 °C and protected from light.

Shelf life

12 months for liquid and dry powdered media.

Do not use after expiration date

Reconstitution method to prepare 1 L EX-CELL® CD Insect Cell Medium

1. Add 800 mL of Milli-Q® or similar cell culture grade water in an appropriately sized container.
2. Add 2 mL of 5N NaOH and stir for 5 minutes.
3. Add 31.7 g of EX-CELL® CD Insect Cell Medium (dry powdered) and stir for 5 minutes.
4. Add 0.760 g of sodium bicarbonate.
5. Add cell culture grade water to reach out 950 mL and stir for 30 minutes.
6. Adjust pH to 6.0 ± 0.1 with 5N HCl.
7. Measure the osmolality of the solution. Final osmolality should be at 380 ± 10 mOsmol/kg. Adjust with NaCl if necessary.
8. Add cell culture grade water to reach a final volume of 1,000 mL.
9. Sterile filter using a sterilizing-grade filter (≤ 0.22 µm).

Initiating Cultures

1. Aseptically add 5 mL EX-CELL® CD Insect Cell Medium to a 125 mL disposable, vented, non-baffled shake flask in a biological safety cabinet.
2. The medium can be used directly from the refrigerator without pre-warming to room temperature. However, if you choose to pre-warm the medium, either place on the counter at room temperature or in a 27-28°C incubator.
3. Quickly thaw a cryovial of frozen cells in a water/bead bath or similar method until just a sliver of ice remains.
4. In the biological safety cabinet, immediately transfer contents of the vial to the shake flask (vented cap) by careful pipetting with a disposable pipette. Swirl culture flask gently to mix.
   - If the cap is not vented, it must be partially unscrewed to allow gas exchange.
   - In this case, loosen cap ½ turn and fix it in place with a piece of masking tape before placing the flask in the shaker.
   - If this is not done, the cap will either come off or close due to the rotating action of the shaking platform.
5. Count the cells and adjust cells the volume to give a viable cell density of around 1 x 10^6 cells/mL.
6. Incubate at 27-28°C with an agitation of 130-140 rpm on a 25-50 mm throw platform. No CO_2 or humidity is required.
7. Count the cells 48 hours post-thaw to culture.

Culturing

1. Swirl to evenly mix the cell culture and remove a sample from the shake flask in the biological safety cabinet.
2. Measure viability and cell density.
3. Observe sample under the microscope to assess cell health.
   - Rounded cells and doublets indicate good cell health
   - Blebbing and debris indicate the cells are unhealthy
4. Passage the cells to 0.5-1 x 10^6 viable cells/mL when they reach 3-6 x 10^6 viable cells/mL in fresh EX-Cell® CD Insect Cell Medium to a volume of 20-50 mL in a 125 mL shake flask.
5. Incubate the flask for 3-4 days (72-96 hours), then passage again. If desired, sample cells for cell counting in between passages.

Cryopreservation

1. Prepare the desired quantity of cells (10 x 10^6 cells/mL), harvesting in mid-logarithmic phase of growth with densities around 4-5 x 10^6 cells / mL and viabilities at >95%.
2. Prepare an insect cell freezing medium by mixing EX-CELL® CD Insect Cell Medium with 10% DMSO (v/v).
3. Harvest cells by centrifugation at 200 x g for 3 minutes. Remove supernatant.
4. Resuspend cells in the insect cell freezing medium from step 2.
5. Rapidly transfer 1 mL of cell suspension to a 2 mL cryovial and cap tightly.
6. Place vials immediately in cryovial freezing container prechilled at 4°C and freeze cells at a rate of 1°C per minute.
7. For long-term storage (>1 month), transfer the vials to liquid nitrogen (vapor phase).

Ordering Information

Product	Cat. No.
EX-CELL® CD Insect Cell Medium	
EX-CELL® CD Insect Cell Medium - Dry Powder	**24381C**
EX-CELL® CD Insect Cell Medium - Liquid	**14380C**
Related Product	
Sodium Bicarbonate	**S5761**
SyntheChol®	**S5442**
Sf-RVN® Insect Cell Line	**SFRVN-1VL**

For additional information, please visit **SigmaAldrich.com**
To place an order or receive technical assistance, please visit **SigmaAldrich.com/customer-service**

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