CD MDBK 211

Version 1.0

CD MDBK 211 is a chemically defined medium designed to support high-density growth of Madin-Darby Bovine Kidney (MDBK) cells in suspension culture and high-efficiency production of bovine viral vaccine.

Product	Catalog No.	Form	Package Sizes
CD MDBK 211	10501-211	Dry powder	5 L, 50 L, 100 L, Customized

Component Information

With glucose and L-glutamine.

Safety Warning

Read the Material Safety Data Sheets (MSDS) and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Intended Use

For research and further manufacturing use only.

Storage and Stability

The dry powder medium should be stored at 2–8°C and protected from light. The product is stable for 24 months, when unopened and stored properly. This product is very hygroscopic and should be kept in a dry environment away from moisture.

Prepare Liquid Medium from Dry Powder

1. Fill the mixing container with purified water (20–30°C) at 80%–90% of the final volume.
2. Slowly add 26.69 g/L of dry powder medium with gentle stirring. Mix for 30 minutes.
3. Slowly add 4.4 mL/L of TE 018 (LP16028).
4. Adjust the pH to 6.8–7.0 using 1 M–5 M NaOH or HCl. Mix for 10 minutes. The pH may rise 0.1 to 0.2 after sterile filtration.
5. Adjust the final volume with purified water. Mix for 10 minutes.
6. Filter the media using a membrane filter with 0.22 μm pore size immediately.

Culture Conditions

Culture Type: Suspension
Culture Vessels: TPP tubes and shake flasks
Shaking Speed: For TPP tubes, it is recommended 200 rpm @50 mm orbital diameter;
For shake flasks, it is recommended 125–150 rpm @25 mm orbital diameter or 90–120 rpm@50mm orbital diameter.
Culture Temperature: 37°C
CO_2 Concentration: 5%
Relative Humidity: 80% RH

Recover Cells

1. Quickly thaw (1–2 minutes) the cryopreserved tube in a 37°C water bath.
2. Transfer all cell fluids into a sterile centrifuge tube with 30 mL prewarmed CD MDBK 211, centrifuge the cells at 1000 rpm (about 233 g) for 5 minutes, discard the supernatant and resuspend the cells with required volume of prewarmed CD MDBK 211 to make sure a final density of 1.0–1.5 × 10^6 cells/mL, then transfer them into a new TPP tube.
3. Culture the cells referring to the “Culture Conditions”.
4. Determine the cell density and viability and add 10 mL CD MDBK 211 at hour 24 ± 2 and 48 ± 2 of first passage respectively.
5. Passage cells every 72 ± 2 hours according to steps in “Passage Cells and Scale-up”.

Passage Cells and Scale-up

1. Determine the cell density and viability at 72 ± 2 hours after recovery.
2. Passage cells by seeding at 0.8–1.2 × 10^6 cells/mL when the viable cell density reach at least 4.5 × 10^6 cells/mL with > 95% viability, then culture the cells referring to the “Culture Conditions”.
3. Before using for other purposes, cells should be passaged at least for 3 times in CD MDBK 211.

Cryopreservation

It is recommended to use cells in mid-logarithmic growth phase (about 72 ± 2 hours after seeding) with > 95% viability for cryopreservation.

1. Prepare cryopreservation medium with 60% CD MDBK 211 + 30% serum + 10% DMSO and store at 4°C until use.
   Note: Add DMSO finally to avoid turbidity.
2. Determine the cell density and viability, and calculate the required volume of cell cultures to give a final cryopreservation density of 30.0 × 10^6 cells/mL.
3. Centrifuge the cells at 1000 rpm (about 233 g) for

5 minutes and discard the supernatant, then resuspend the pellet in the pre-determined volume of cryopreservation medium.
4. Aliquot the suspension into 1 mL per cryovial, then transfer them into a pre-cooled freezing container immediately and place at −80°C for 24 hours.
5. Transfer frozen cells to liquid nitrogen for long-term storage.

Switch from Adherent to Suspension Culture in CD MDBK 211

1. Seed MDBK cells dissociated from adherent condition at 1.0–2.0 × 10^6 cells/mL in CD MDBK 211, then culture the cells referring to the “Culture Conditions”.
2. Resuspend pallet after centrifuging cells at 1000 rpm (about 233 g) for 5 minutes and discarding the supernatant at 72±2 hours after seeding, then culture the cells referring to the “Culture Conditions”.
3. After several passages in CD MDBK 211, the viable cell density should be reached at least 4.0 × 10^6 cells/mL. At this stage, the cells can be passaged without centrifugation by seeding at 0.8–1.0 × 10^6 cells/mL.

Produce Bovine Viral Diarrhea Virus (BVDV)

The following method is used for early production only. Optimization of seeding process is encouraged to obtain higher virus titer.

1. The viable cell density before infection should be reached 5–7 × 10^6 cells/mL with > 95% viability.
2. Dilute cell density with CD MDBK 211 to 2.5–3.5 × 10^6 cells/mL.
3. Add BVDV, and culture the cells referring to the “Culture Conditions”.
4. Harvest BVDV according to cytopathic effect (e.g., 48–72 hours post-infection).

Related Products

Product	Catalog No.
Glucose (300 g/L)	99024-16023
L-Glutamine (200 mM)	99025-17029
0.25% Trypsin-EDTA	99008-14020