Celkey® MDCK SFM 1607

Version 1.0

MDCK SFM 1607 Medium is a personalized serum-free medium designed for high-density suspension culture of canine kidney cells (MDCK) and contains no animal-derived ingredients. It is specifically designed for the following purpose:

- Enable rapid adaptation of MDCK cells from adherent to suspension culture.
- Support high-density growth of the adapted suspension cells.
- Promote efficient viral expression, such as that of AIV.

Product Name	Catalog No.	From	Product Series	Product Usage
MDCK SFM 1607	10405-1607	Dry powder	Serum Free Media	Cell Growth & Virus Expression
MDCK SFM 1607	10405-24009	Liquid	Serum Free Media	Cell Growth & Virus Expression

Note: MDCK SFM 1607 Medium is available in two formulations: a dry powder (Catalog No. 10405-1607) and a ready-to-use liquid (Catalog No. 10501-24009).

Component Information

With glucose and L-glutamine.

Safety Warning

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

Intended Use

For research and further manufacturing use only.

Storage and Stability

Liquid Medium

The liquid medium should be stored at 2–8℃ and protected from light. It has a shelf life of 1 month. The addition of other supplements may affect storage conditions and shelf life. If precipitation or turbidity occurs, discontinue use.

Powder Medium

Store the dry powder medium at 2–8℃ and protect from light and moisture. The unopened product is stable for 24 months when stored properly. This product is highly hygroscopic; ensure the container is kept tightly sealed in a dry environment.

Prepare Liquid Medium from Dry Powder

Prepare in accordance with the product preparation instructions.

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@50 mm orbital diameter.
- **Culture Temperature:** 37℃
- **CO_2 Concentration:** 5%
- **Relative Humidity:** 80% RH

Recover Cells

- **Thawing:** Rapidly thaw the cryopreserved vial in a 37℃-water bath for 1 to 2 minutes.
- **Centrifugation and Resuspension:**
  a. Aseptically transfer the entire cell contents into a sterile centrifuge tube containing 30 mL of pre-warmed MDCK SFM 1607 medium.
  b. Centrifuge at 1,000 rpm (approximately 233 × g) for 5 minutes.
  c. Carefully discard the supernatant.
  d. Resuspend the cell pellet in a required volume of pre-warmed MDCK SFM 1607 medium to achieve a final seeding density of (1.0–1.5) × 10^6 cells/mL.
  e. Transfer the cell suspension into a new TPP tube or shaker flask.
- **Initial Culture:** Incubate the cells under the conditions specified in the "**Culture Conditions**" section.
- **Routine Passaging:** Beginning with the first full passage, subculture the cells every 48 ± 2 hours by following the steps outlined in "**Passage Cells and Scale-up**".

Passage Cells and Scale-up

- **Pre-Passage Assessment:** At 48 ± 2 hours post-recovery or post-previous passage, determine the cell density and viability.
- **Passaging Criteria and Execution:**
  a. When the viable cell density reaches at least 7.0 × 10^6 cells/mL with viability exceeding 95%, subculture the cells by seeding them at a density of (1.2–1.5) × 10^6 cells/mL in fresh MDCK SFM 1607 medium. If the viable cell density is less than 4.0 × 10^6 cells/mL or cell viability drops below 95%, perform centrifugal subculture: centrifuge the cell suspension at 1,000 rpm (approximately 233 × g) for 5 minutes, discard the supernatant, and subculture at a seeding density of (1.2 ± 1.5) × 10^6 cells/mL.
  b. Culture the newly seeded cells according to the "**Culture Conditions**".
- **Adaptation Requirement:** Prior to use in other applications, cells must undergo a minimum of 3 consecutive, successful passages in MDCK SFM 1607 medium to ensure proper adaptation.

Adaptation to MDCK SFM 1607 Medium

Direct Adaptation

- **Initial Seeding:** Directly transfer cells from their current medium into MDCK SFM 1607 medium at a seeding density of (1.2–1.5) × 10^6 cells/mL. Culture the cells according to the specifications in the "Culture Conditions" section.
- **Monitoring:** Following the initial transfer, it is recommended to monitor cell density (× 10^6 cells/mL) and viability (%) daily.
- **Success Criterion:** Adaptation is considered successful when, after several consecutive passages, the cell growth profile (including doubling time and maximum density) aligns consistently or beyond with the historical data from the client's existing process.
- **Passaging and Other Experiments:**
  a. Perform routine passaging by following the procedures outlined in "**Passage Cells and Scale-up**".

b. Cells should undergo a minimum of 3 to 5 consecutive, stable passages in MDCK SFM 1607 medium before used for other experiments.

Sequential Adaptation

- **Initial Seeding:** Begin by seeding MDCK cells at a viable cell density of (1.2–1.5) × 10^6 cells/mL in a mixture of the original medium and MDCK SFM 1607 medium.
- **Gradual Media Transition:** During each subsequent passage, gradually reduce the proportion of the original medium while increasing that of MDCK SFM 1607 medium, transitioning sequentially through the following ratios: 90:10 (Passage 1) → 75:25 (Passage 2) → 50:50 (Passage 3) → 25:75 (Passage 4) → 0:100 (Passage 5, representing 100% MDCK SFM 1607).
- **Process Alignment & Verification:**
  a. Once the cell growth profile demonstrates alignment with the trend of the client's established process, conduct an additional 2 to 3 passages under the same conditions.
  b. Proceed to the next stage only if consistent and reproducible growth is maintained throughout this verification period.
- **Final Adaptation in 100% Medium:** Continue passaging the cells in 100% MDCK SFM 1607 medium. Successful adaptation is confirmed when the growth profile consistently matches or beyond the client's process trend, which typically requires a minimum of 3 to 5 successful passages in the 100% medium. Upon meeting this criterion, the cells are ready for use in other experiments.

Cryopreservation

- **Recommended Cell State：**
  Use cells in mid-logarithmic growth phase (approximately 48 ± 2 hours of post seeding) with viability > 95%.
- **Procedure：**
  Prepare cryopreservation medium (60% MDCK SFM 1607 + 30% serum + 10% DMSO) and store at 2–8℃ until use.
  a. Determine cell density and viability, then calculate the volume needed to obtain a final concentration of 2.5 × 10^7 cells/mL.
  b. Harvest cells by centrifugation (1,000 rpm ≈ 233 × g, 5 min), discard supernatant, and resuspend the pellet in the pre-calculated volume of chilled cryopreservation medium.
  c. Aliquot 1 mL per cryovial, place vials into a pre-cooled freezing container, and transfer to a -80℃ freezer for ≥ 24 hours.
  d. For long term storage, transfer frozen vials to the vapor phase of liquid nitrogen.

Virus Production

- **Scope**
  This section outlines the baseline method for initial virus production. Process optimization (e.g., seeding density, infection timing, harvest time) is recommended to achieve higher titer.
- **Pre-infection Cell Preparation**
  a. Culture cells to a pre-infection density of (7.0–9.0) × 10^6 cells/mL with viability > 95%.
  b. Dilute the culture with fresh MDCK SFM 1607 medium to the target infection density specified below.
- **Infection & Harvest Protocol**

Step	Target Density	Infection	Incubation
Parameter	Seeding density for infection	Multiplicity of Infection (MOI)	Culture conditions
Avian Influenza Virus (AIV)	(2.5–5.0) × 10^6 cells/mL	0.0001–0.01	It is recommended to conduct the cultivation of AIV, SIV, CPV, CDV, CAV-2, and CPIV at a reduced temperature
Swine Influenza Virus (SIV)	(2.5–5.0) × 10^6 cells/mL	0.01–0.1	

Canine Parvovirus (CPV)	(2.5–5.0) × 10^6 cells/mL	0.001–0.1	range of 33–35℃. Other culture parameters. refers to "Culture Conditions"
Canine Distemper Virus (CDV)	(2.5–5.0) × 10^6 cells/mL	0.001–0.01	
Canine Adenovirus Type 2 (CAV-2)	(2.5–5.0) × 10^6 cells/mL	0.01–0.1	
Canine Parainfluenza Virus (CPIV)	(2.5–5.0) × 10^6 cells/mL	0.01–0.1	

- **Preparation and Use of Viral Additives：** To enhance viral replication efficiency in MDCK suspension cells, the addition of TPCK-Trypsin to the culture system is recommended during the infection stage. Recommended Concentration: 5–10 mg/L (final concentration).

- **Primary Harvest Criterion:** Determine the optimal harvest time based on cytopathic effect (CPE) and virus titer assay. Furthermore, the recommended harvest window for these viruses typically falls within 48–120 hours post-infection.
- **Typical Harvest Windows (for reference):**
  a. **AIV: 48–120 hours post-infection.**
  b. **SIV: 48–72 hours post-infection.**
  c. **CPV: 48–72 hours post-infection.**
  d. **CDV: 48–120 hours post-infection.**
  e. **CAV-2: 48–96 hours post-infection.**
  f. **CPIV: 48–96 hours post-infection.**