Celkey® F81 LSM 801

Version 1.0

F81 LSM 801 is a low-serum medium specifically designed to:

- Enable rapid adaptation of F81 cells from adherent to suspension culture.
- Support high-density growth of the adapted suspension cells.
- Promote efficient viral expression for pet virus vaccine production, such as Feline Herpesvirus-1 (FHV-1), Feline Panleukopenia Virus (FPV), and Feline Calicivirus (FCV).
- Be used with a standard 3% serum supplementation for experimental procedures.

Product Name	Catalog No.	Form	Product Series	Product Usage
F81 LSM 801	11401-801	Dry powder	Low-Serum Media	Cell Growth & Virus Expression

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

Store the liquid medium at 2–8℃ and protect from light. The unopened product is stable for 12 months when stored under these conditions. The addition of supplements may alter storage requirements and shelf life. Do not use if particulate matter or cloudiness is observed.

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:** Shake flasks
- **Shaking Speed:** 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 suspension into a sterile centrifuge tube containing 20 mL of pre-warmed F81 LSM 801 medium supplemented with 3% NBCS.
  b. Centrifuge at 1000 rpm (approximately 233 × g) for 5 minutes.
  c. Carefully discard the supernatant.
  d. Resuspend the cell pellet in an appropriate volume of pre-warmed F81 LSM 801 medium supplemented with 3% NBCS to achieve a final seeding density of (0.8–1.5) × 10^6 cells/mL.
  e. Transfer the cell suspension into a new shake flask.
- **Initial Culture:** Incubate the cells under the conditions specified in the "**Culture Conditions**" section.
- **Routine Passaging:** Beginning with the first full passage after recovery, subculture the cells every 72 ± 2 hours by following the steps outlined in "**Passage Cells and Scale-up**".

Passage Cells and Scale-up

- **Pre-Passage Assessment:** At 72 ± 2 hours post-recovery or post-previous passage, determine the cell density and viability.
- **Passaging Criteria and Execution:**
  a. hen the viable cell density reaches at least 4.0 × 10^6 cells/mL with viability exceeding 95%, subculture the cells by seeding them at a density of (1.5 ± 0.2) × 10^6 cells/mL in F81 LSM 801 medium supplemented with 3% NBCS. If the viable cell density is < 4.0 × 10^6 cells/mL or the cell viability is < 95%, perform centrifugal subculture by centrifuging the cell suspension at 233 × g (approximately 1000 rpm) for 5 minutes, discarding the supernatant, and subculturing at a seeding density of (1.5 ± 0.2) × 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 F81 LSM 801 medium supplemented with 3% NBCS to ensure proper adaptation.

Adaptation to F81 LSM 801 Medium

Direct Adaptation

- **Initial Seeding:** Directly transfer cells from their current medium into F81 LSM 801 medium supplemented with 3% NBCS at a seeding density of (1.5 ± 0.2) × 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 the viable 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 with or exceeds 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 F81 LSM 801 medium supplemented with 3% NBCS before being used for other experiments.

Sequential Adaptation

- **Initial Seeding:** Begin by seeding F81 cells at a viable cell density of (1.5 ± 0.2) × 10^6 cells/mL in a mixture of the original medium and F81 LSM 801 medium supplemented with 3% NBCS.
- **Gradual Media Transition:** During each subsequent passage, gradually reduce the proportion of the original medium while increasing that of F81 LSM 801 medium supplemented with 3% NBCS, 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% F81 LSM 801 medium supplemented with 3% NBCS).

- **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% F81 LSM 801 medium supplemented with 3% NBCS. Successful adaptation is confirmed when the growth profile consistently matches or exceeds 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.
  Note：The section "**Adaptation to F81 LSM 801 Medium**" applies only when transferring the customer's cells into F81 LSM 801 Medium.

Cryopreservation

- **Recommended Cell State：**
  Use cells in mid-logarithmic growth phase (approximately 72 ± 2 hours post seeding) with viability > 95%.
- **Procedure：**
  Prepare cryopreservation medium (50% F81 LSM 801 + 40%NBCS + 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 the 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 h.
  d. For long-term storage, transfer frozen vials to the vapor phase of liquid nitrogen.

Virus Production (FHV-1, FPV, FCV)

- **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 (4.0–6.0) × 10^6 cells/mL with viability > 95%.
  b. Dilute the cell suspension to (2.0–2.5) × 10^6 cells/mL and transfer it to the appropriate culture vessel.
- **Infection Protocol**

Step	Parameter	FHV-1, FPV, FCV
Target Density	Seeding density for infection	(2.0–2.5) × 10^6 cells/mL
Infection	Multiplicity of Infection (MOI)	0.1–1.0
Incubation	Culture Conditions	Refer to "**Culture Conditions**"

- **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.