gibco
by life technologies

OptiPRO™ SFM

Description

OptiPRO™ SFM is a chemically defined serum-free, animal/human-origin free medium, designed for growth of several kidney-derived cell lines including MDCK, MDCF, VERO, BHK-21, and PK-15 which are important for virus and recombinant protein production. OptiPRO™ SFM has also been successfully used to grow several additional attachment dependent cell lines including COS-7, MDBK, and HeLa cells. In addition no adaptation to OptiPRO™ SFM is necessary for many cell lines. OptiPRO™ SFM is formulated without L-glutamine for greater stability and extended shelf life.

Product	Catalog no.	Amount	Storage	Shelf Life*
OptiPRO™ SFM (1X), liquid	12309-019 12309-050	1000 mL 100 mL	2°C to 8°C; Protect from light	24 months

* Shelf Life duration is determined from Date of Manufacture.

Product use

Caution: For manufacturing, processing, or repacking.

Important information

- Ultra-low protein concentration ≤7.5 µg/mL.

Safety information

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

Prepare medium

- OptiPRO™ SFM medium requires aseptic supplementation with L-glutamine or GlutaMAX™-I to 4 mM final concentration (20 mL/L), prior to use.
- Antibiotics are not recommended; however, 5 mL/L of Antibiotic-Antimycotic (100X) containing penicillin, streptomycin, and amphotericin B may be used when required.

Culture conditions

Media: Supplemented OptiPRO™ SFM.

Cell Line(s): MDCK, MDCF, VERO, BHK-21, PK-15, COS-7, MDBK, and HeLa.

Culture Conditions: Adherent

Culture Vessels: T-75 flasks.

Temperature Range: 36°C to 38°C.

Incubator Atmosphere: Humidified atmosphere of 5–8% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a tissue culture flask containing 15 mL pre-warmed supplemented OptiPRO™ SFM.
3. Incubate at 36°C to 38°C in a humidified atmosphere of 5-8% CO_2 in air. Loosen flask caps to allow for gas exchange.
4. Subculture cells 1-3 days post thaw when cells reach 70-90% confluence.

Subculture cells

Ensure that the cell confluency is between 70–90%, cell viability is at least 90%, and growth rate is in mid-logarithmic phase prior to subculturing. **Note:** Procedures are for cultures in a T-75 cm^2 flask. Adjust volumes accordingly to culture vessel size.

1. Observe cell monolayer to ensure confluence (70–90%). Aspirate medium and floating cells from monolayer and discard.
2. Add 5–10 mL Dulbecco's Phosphate Buffered Saline (DPBS), without calcium and magnesium to culture flask. Gently wash the cell monolayer.
3. Remove DPBS and add 5–7 mL of prewarmed TrypLE™ Select (without phenol red) to the monolayer.
4. After 2 minutes, remove the TrypLE™ Select and incubate flask at 37°C for approximately 10–15 minutes or until cells have fully detached. Observe cell monolayer using an inverted microscope to ensure complete cell detachment from the surface of the flask.
5. Add 5–7 mL of prewarmed supplemented OptiPRO™ SFM to the flask to resuspend the cells.
6. Disperse cell clusters into a single-cell suspension by triturating with a small bore pipette or vortexing before passaging or counting. Optimal vortexing conditions must be determined based upon speed and duration versus viability.
7. Determine viable cell density using a Countess® Automated Cell Counter. Alternate methods (e.g., Coulter counter or hemocytometer) may also be used.
8. Inoculate flask at 1–4 × 10^4 viable cells/cm^2.
9. Incubate at 37°C in a humidified atmosphere of 5–8% CO_2 in air.

For optimal performance and cell growth, re-feed cultures every 3–4 days with fresh medium. Subculture cells when confluency reaches 70–90%.

Adapt cultures to OptiPRO™ SFM

For many cell lines grown in conventional 5–10% serum supplemented medium or other serum-free medium little or no adaptation is needed and may be directly converted to OptiPRO™ SFM. It is advisable to keep a backup culture in the original media until cells have adapted. If suboptimal growth is observed after direct adaptation for 3–5 passages use the sequential adaptation method.

Sequential adaptation

1. Subculture cells into a 25:75 ratio of supplemented OptiPRO™ SFM to the original media. During the adaptation procedure seed at twice the normal seeding density ($2–8 \times 10^4$ viable cells/cm^2).
2. Subculture cells when confluency reaches 70–90%. Subculture the cells in fresh prewarmed 25:75 ratio of supplemented OptiPRO™ SFM to the original media. Once consistent cell growth with high viability has been achieved, passage cells into a 50:50 ratio of supplemented OptiPRO™ SFM to original medium.
3. Repeat step 2 of this procedure, stepwise increasing the ratio of OptiPRO™ SFM to original medium (75:25 followed by 90:10) until the cells are subcultured into 100% OptiPRO™ SFM. Multiple passages at each step may be needed.
4. Continue to monitor and passage cells until consistent growth with high viability is achieved. After several passages in 100% OptiPRO™ SFM, the culture is considered to be adapted.

Cryopreservation

1. Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of $1–5 \times 10^6$ cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% OptiPRO™ SFM (50:50 fresh to conditioned OptiPRO™ SFM) + 7.5% DMSO, and store at 4°C until use. IMPORTANT! Prepare cryopreservation medium on the day of intended use.
4. Centrifuge cells, harvested in step 1 of this procedure, at $100 \times g$ for 5–10 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase); storage at –200°C to –125°C is recommended.

Related products

Product	Catalog no.
L–Glutamine-200mM (100X), Liquid	25030
GlutaMAX™-I, 200mM (100X), Liquid	35050
Antibiotic-Antimycotic (100X), Liquid	15240
Dulbecco's Phosphate Buffered Saline (DPBS), without calcium and magnesium	14190
TrypLE™ Select (1X), without Phenol Red	12563
0.25% Trypsin-EDTA (1X), Phenol Red	25200
Trypsin Inhibitor, soybean	17075
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

MM-YYYY		LOT		
Use By:	Manufacturer	Batch code	Keep away from light	Temperature Limitation
REF				STERILE A
Catalog number	Consult instructions for use		Caution, consult accompanying documents	Sterilized using aseptic processing techniques

Limited Use Label License: Internal Research and Bioproduction Use

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