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by life technologies

VP-SFM

Description

VP-SFM is a serum-free, ultra-low protein (5 µg/mL) medium containing no proteins, peptides, or other components of animal or human origin. It is designed for the growth of VERO cells for virus production. VP-SFM is also suitable for the growth of COS-7, MDCK, BHK-21 (suspension culture) and HEp2 cells, providing equivalent growth, virus titer and recombinant protein production to serum supplemented media.

Product	Catalog no.	Amount	Storage	Shelf Life*
VP-SFM (1X), liquid	11681-020	1000 mL	2°C to 8°C; Protect from light	12 months
VP-SFM AGT™**	12559-027	1 × 1 L	2°C to 8°C; Store dark and dry	24 months
	12559-019	1 × 10 L	2°C to 8°C; Store dark and dry	24 months
	12559-001	1 × 100 L	2°C to 8°C; Store dark and dry	24 months
	12559-003	1 × 10 kg	2°C to 8°C; Store dark and dry	24 months

* Shelf Life duration is determined from Date of Manufacture.
**AGT= Advanced Granulation Technology.

Product Use

Caution: For manufacturing, processing, or repacking.

Safety Information

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

Reconstitute VP-SFM AGT™:

1. Measure distilled water to 90% of final volume into an appropriately sized clean vessel.
2. Add VP–SFM AGT™ Medium at 17.6 g/L. Mix for 30minutes or until completely dissolved, **do not heat**.
3. Add distilled water to the desired final volume.
4. Filter sterilize by 0.2 µm pore size membrane filtration.
   Note: Use low protein binding, low extractables filter.

Note: VP-SFM AGT™ Medium contains sodium bicarbonate. **Do not** add additional sodium bicarbonate. VP-SFM AGT™ Medium is auto pH and osmolality adjusted, no further adjustment required. For final lot pH and osmolality specifications refer to the Certificate of Analysis available from our website, **www.lifetechnologies.com/support**.

Prepare Media

- VP-SFM and VP-SFM AGT™ Medium require aseptic supplementation with 4 mM L-glutamine or GlutaMAX™-I (20 mL/L), prior to use.
- The use of antibiotics is not recommended; however, addition of 0.5X Antibiotic-Antimycotic containing penicillin, streptomycin and amphotericin B may be used if desired.
- Once supplemented, the complete VP-SFM should be stored at 2°C to 8°C protected from light.

Culture Conditions

Media: complete VP-SFM

Cell Line(s): VERO, COS-7, MDCK, BHK-21, and HEp2

Culture Type: Adherent

Culture Vessels: T-75 flask

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 T-75 flask containing 15 mL of prewarmed complete VP-SFM medium without antibiotics.
3. Incubate at 37°C in a humidified atmosphere of 5–8% CO_2 in air. Loosen flask caps (or use vented caps) to allow for gas exchange.
4. Subculture when cells reach 80–100% confluency (1–3 days post thaw). Subculture cells a minimum of 3 passages before use in other applications.

Subculturing in VP-SFM

1. Aspirate Medium from cell monolayer and rinse flask with 5 mL prewarmed DPBS without Ca^{2+} or Mg^{2+}. Aspirate DPBS.
2. Add 5 mL prewarmed TrypLE™ Select or 0.25% Trypsin-EDTA to flask (1 mL for a T-25 cm^2 flask).
3. Incubate until cells have detached (~2–5 minutes at room temperature). Gently tap flask to dislodge cells.
4. Stop the dissociation reaction by adding 9 mL of complete VP-SFM growth medium to the flask. Tilt flask in all directions to thoroughly rinse flask.
   Note: If using 0.25% Trypsin-EDTA, addition of 500 µg/mL Soybean Trypsin Inhibitor is required.
5. Transfer cell suspension to a sterile 15-mL centrifuge tube and centrifuge at 100 × *g* for 5 minutes.
6. Aspirate supernatant and resuspend the cell pellet in 10 mL complete VP-SFM growth medium. If cell clumping is observed disperse cells by pipetting up and down or vortexing until clumps are dispersed into a single cell suspension. Optimal vortexing conditions must be determined based upon speed and duration versus viability.
7. Determine total viable cell density using a Countess® Automated Cell Counter (alternate automated or manual methods may be used).
8. Seed flasks at 1–5 × 10^4 viable cells/cm^2.
9. Incubate at 37°C in a humidified atmosphere of 5–8% CO_2 until cells are 80–100% confluent, usually 3–5 days post-plating.

Adapt Cultures to VP-SFM

A sequential adaptation protocol may be necessary, however, for many cell lines, such as VERO, MDBK, BHK-21 (grown in suspension culture) and HEp2, little or no adaptation is needed.

Direct Adaptation

It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation. Successful adaptation will depend upon the particular cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

1. Subculture cells grown in conventional medium with 5–10% serum or other serum-free medium into prewarmed complete VP-SFM. During the adaptation procedure seeding density should be double the normal seeding density for the cell line for the first two or three passages.
2. Continue to monitor and passage cells for 3–5 passages until consistent growth is achieved.

Note: If suboptimal performance is observed using the direct adaptation method over 3–5 passages, use the sequential adaptation method.

Sequential Adaptation

1. Subculture cells grown in conventional medium with 5–10% serum or other serum-free medium into a 25:75 ratio of complete VP-SFM to the original media. During the adaptation procedure seed at double the normal seeding density.
2. When consistent growth and high viability (>90%) is observed subculture cells into a 50:50 ratio of complete VP-SFM to original medium.
3. Repeat step 2, stepwise increasing the ratio of VP-SFM to original medium (75:25 followed by 90:10) until the cells are transferred into 100% VP-SFM. Multiple passages at each step may be needed.
4. Continue to monitor and passage cells until consistent growth and high viability (>90%) is achieved. After several passages of consistent growth and viability in 100% complete VP-SFM the culture is considered to be adapted.

Cryopreservation

Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%. Save the conditioned medium to prepare cryopreservation medium.

1. Prepare desired quantity of cells in T-flask cultures, harvesting when cells reach approximately 80% confluency.
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% VP-SFM (50:50 ratio of fresh to conditioned media) +7.5% DMSO on day of intended use, and store at 4°C until use.
   Note: Conditioned medium should be from day 2–4 cultures collected prior to subculture and trypsinization procedure.
4. Harvest cells (see *Subculturing in VP-SFM* steps 1–4) and centrifuge at 100 × *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.

Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen (See **Recovery**).

Related Products

Product	Catalog no.
L–Glutamine, 200mM (100X), liquid	25030
GlutaMAX™-I, 200mM (100X), liquid	35050
Antibiotic-Antimycotic (100X), liquid	15240
Penicillin-Streptomycin, Liquid	15140
Fungizone® Antimycotic, Liquid	15290
Dulbecco's Phosphate Buffered Saline (DPBS), without calcium and magnesium	14190
TrypLE™ Select (1X), liquid, without Phenol Red	12563
Trypsin-EDTA, 1X	25300
Trypsin Inhibitor, Soybean	17075
Water, Distilled	15230
Gibco® Water for Injection (WFI) for Cell Culture	A12873
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

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

Limited Use Label License: Internal Research and Bioproduction Use

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) to culture cells for the purpose of producing a product wherein the product will be used for any or all of the following: (i) internal research use by the purchaser; (ii) resale for internal research use by third parties; (iii) performance of research conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; (iv) resale for use as a human therapeutic agent or diagnostics product or component by third parties; (v) performance of manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties.

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**