GIBCO™
Invitrogen Corporation

COS-7L Cells

Cat. No. 11622-016

Shipping and Storage

Cells are supplied in a cryogenic vial containing 7.5 x 10^6 viable cells in a volume of 1.5 mL. **Store in liquid nitrogen (vapor phase).**

Caution

Handle as potentially biohazardous material under at least Biosafety Level 2 containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

General Media Requirements

Use VP-SFM supplemented with 4 mM L-Glutamine. Protect medium from light. Antibiotics are not recommended; however, 5 mL/L of Penicillin-Streptomycin may be used when required.

Thawing Cells

Store frozen COS-7L cells in liquid nitrogen (vapor phase) until ready to use. Frozen cells are supplied in and may be thawed directly into VP-SFM. Use the following procedure to thaw cells.

1. Rapidly thaw frozen vial in a 37°C water bath. Triturate and transfer the entire contents of the cryovial into a T-75 cm^2 flask containing 18 mL of pre-warmed VP-SFM supplemented with 4 mM L-Glutamine, and incubate in a 37°C incubator containing a humidified atmosphere of 5% CO_2 in air. Loosen caps of flasks to allow oxygenation/aeration.
2. Once the flask has reached 80-100% confluency, subculture cells using seeding densities of 1 to 2 x 10^4 viable cells/cm^2 (see **Subculturing Cells** for details).

Note: Upon recovery from thawing, COS-7L cells may grow in loosely attached clumps for 1 to 3 passages. If cells grow in this fashion, replace media every 3 to 4 days with fresh growth media until the cells are >60% confluent, then subculture cells using the **Subculturing Procedure**. We recommend subculturing cells for a minimum of 3 passages before use in other applications.

Determining Cell Density and Viability

Follow the procedure below to determine viable and total cell counts.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.
2. Determine viability and the amount of cell clumping using the trypan blue exclusion method.
3. Vigorously vortex cells for up to 40 seconds to break up cell clumps.
4. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

Subculturing Cells

Recommended Conditions

Cell density	80-100% confluent
Culture vessel	T-75 cm^2 to T-162 cm^2 disposable sterile T-flasks. Dilute cells in a total working volume of 15-20 mL for T-75 cm^2 flasks and 40-50 mL for T-162 cm^2 flasks
Seeding density	1 to 2 x 10^4 viable cells/cm^2
Incubation conditions	37°C incubator with a humidified atmosphere of 5% CO_2 in air; loosen caps to allow for oxygenation/aeration

Subculturing Procedure

Use this procedure to subculture COS-7L cells grown in a T-75 cm^2 flask. If you are using other-sized flasks, scale the reagent volumes up or down accordingly. Note that complete VP-SFM is VP-SFM supplemented with 4 mM L-Glutamine.

1. Remove media from the flask. Rinse the flask with 5 mL of Dulbecco's Phosphate-Buffered Saline (D-PBS) without Ca^{2+} or Mg^{2+} and remove.
2. Add 1 mL of pre-warmed 0.25% Trypsin-EDTA to the flask.
3. Incubate until the cells have detached (about 2 to 5 minutes at room temperature).
4. Add 9 mL of complete VP-SFM containing 500 µg/mL Soybean Trypsin Inhibitor to the flask and transfer the cell suspension to a 15mL centrifuge tube.
5. Centrifuge for 5 minutes at 100 x g.
6. Aspirate the supernatant and resuspend the cell pellet in 10 mL of complete VP-SFM.
7. Determine viable and total cell counts (see procedure).
8. Seed cells at the recommended density (see table), diluting in pre-warmed growth medium. Put flasks in incubator with caps loosened to allow for oxygenation/aeration.

Freezing Cells

Recommended Conditions

- Freeze cells at a density of ≥5 x 10^6 viable cells/mL.
- Use a freezing medium composed of 50% fresh growth medium and 50% conditioned growth medium (day 2 to 4 cell conditioned media collected from COS-7L cultures prior to the subculture procedure) and DMSO to a final concentration of 7.5%. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and chill at 4°C until use. Discard any remaining freezing medium after use.

Freezing Procedure

1. Grow the desired quantity of COS-7L cells in T-flasks, harvesting when the flasks are 80-100% confluent. Follow **Subculturing Procedure**, Steps 1-6.
2. Determine viable and total cell counts (see procedure) and calculate the volume of freezing medium required to yield a final cell density of ≥5 x 10^6 viable cells/mL.
3. Prepare the required volume of freezing medium (see above).
4. Centrifuge cells from cell suspension (Step 1) at 100 x g for 5 minutes. Aseptically decant supernatant and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
5. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications (*i.e.* 1.5 mL in a 2 mL cryovial).
6. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
7. Transfer frozen vials to liquid nitrogen (vapor phase) storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing vials in liquid nitrogen by following the procedure outlined in **Thawing Cells**.

Transfection

For optimal results, we recommend using LIPOFECTAMINE™ 2000 available from Invitrogen for transfection (see the **Transfection Note** on the next page for more information). Refer to the manual accompanying the product for instructions. Other transfection reagents are suitable.

Transfection Note: VP-SFM can inhibit complex formation of DNA with some transfection reagents (*e.g.* LIPOFECTAMINE 2000, LIPOFECTAMINE™, and Lipofectin®). If you are using one of these transfection reagents, culture cells in another medium immediately prior to and during the transfection. We recommend transferring cells from VP-SFM to serum-supplemented media (*i.e.,* Dulbecco's Modified Eagle Medium supplemented with 0.1 mM MEM Non-Essential Amino Acids and 10% Fetal Bovine Serum) several days prior to transfection. Cells should adapt directly into the serum-supplemented media without any trouble. After transfection and selection, COS-7L cells can be expanded and re-adapted back into serum-free culture in VP-SFM supplemented with 4 mM L-Glutamine and the appropriate selective antibiotic.

General Information

The COS-7L cell line is an African green monkey kidney cell line derived from CV-1 simian cells that were transformed by an origin-defective SV40 mutant coding for wild-type Large T antigen[1]. The presence of the Large T antigen in COS-7L cells results in over-expression of heterologous genes whose expression is controlled by the SV40 Large T promoter (*e.g.* pSV2CAT). The COS-7L cell line is a suitable host for infection by recombinant SV40 viruses, and can retain complete permissiveness for lytic growth and support the replication of tsA209 virus at 40°C and pure populations of SV40 mutants with deletions in the early region[1,2].

The COS-7L cell line exhibits the following general features:

- Prepared from low passage Master Cell Bank cultures derived from parental COS-7L cells that were re-cloned by limiting dilution in VP-SFM, and selected for their superior serum-free cell growth and transfection efficiency[3]. The clonal derived cultures are maintained in serum-free conditions for only 20 to 30 total passages.
- Adapted to serum-free growth in VP-SFM, an ultra-low protein containing medium, formulated with no components of animal or human origin, and designed to support serum-free growth of COS-7L and other cells[4]. **Note:** Cells also grow well in traditional media supplemented with serum.

Product Qualification

Frozen catalog COS-7L cells are performance tested for viability and cell growth post-recovery from cryopreservation, and are screened for mycoplasma and sterility. Master Cell Banks are screened for viruses, mycoplasma, and sterility. Species identity is confirmed by isozyme and karyotype analysis.

References

1. ATCC Catalog of Cell Lines and Hybridomas (1992) 7th edition.
2. Glutzman, Y. (1981) *Cell 23*, 175.
3. Ciccarone, V., Chu, Y., Schifferli, K., Pichet, J.P., Hawley-Nelson, P., Evans, K., Roy, L., and Bennett, S. (1999) *Focus 21*, 54.
4. Price, P. and Evege, E. (1997) *Focus 19*, 67.

Related Products

	Quantity	Cat. No.
VP-SFM	1 L	11681-020
200 mM L-Glutamine	20 mL	25030-149
Penicillin-Streptomycin	100 mL	15070-063
Dulbecco's Phosphate-Buffered Saline (D-PBS)	500 mL	14190-144
Trypsin-EDTA	100 mL	25200-056
Soybean Trypsin Inhibitor	1 g	17075-029
Dulbecco's Modified Eagle Medium	500 mL	11965-092
10 mM MEM Non-Essential Amino Acids	100 mL	11140-050
Fetal Bovine Serum	500 mL	26140-079
LIPOFECTAMINE™ 2000	0.75 mL	11668-027

Note: Other reagent sizes are available.

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United States Technical Service: 1 800 955 6288
Canada Technical Service: 1 800 757 8257
Outside the U.S. and Canada: refer to the GIBCO™ products catalog

You may also contact your Invitrogen Sales Representative or visit our World Wide Web site at www.invitrogen.com.

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