Caco-2 Cell Line

Cat. No. : CL-0050

Origin and General Characteristics

Cell Name	Caco-2
Organism	Human
Age	72Y
Sex	Male
Tissue	Colon
Morphology	Epithelial-like
Growth Properties	Adherent
Background	Caco-2 cells were isolated from a primary colonic adenocarcinoma. When grown to confluence, Caco-2 cells exhibit characteristic enterocytic differentiation. They express both cellular retinoic acid-binding protein I and II and are positive for cytokeratin.
Biosafety Level	BSL-1

Culture Conditions and Handling

Complete Medium	MEM, with NEAA[PM150410]+20% Nutrient+1% Supplement
Subcultivation Ratio	1:3-1:6
Medium Renewal	2 to 3 times per week
Dissociation Duration	5-10 min
Incubation Atmosphere	Air, 95%; CO_2, 5%
Temperature	37°C
Freeze Medium	Freezing Medium (Serum-free & animal origin-free)[PB180438]
Storage Conditions	For long-term cryopreservation, cryovials should be stored in liquid nitrogen at −150°C to −196°C. Storage at −80°C is restricted to short-term interim use only.

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Instructions	1. Check all containers for leakage or breakage. 2. Remove the frozen cells from the dry ice packaging and immediately transfer them to liquid nitrogen (liquid or vapor phase) for long-term cryopreservation. 3. Cells exhibit slow adherence, especially shortly after recovery; they generally adhere and spread out within 2–3 days, and vacuoles may be present. 4. Ensure thorough cell dispersion during subculture; otherwise, cells will have difficulty adhering. 5. Cells grow in sheets; the higher the cell density, the more vacuoles and dark granules appear on the cell surface. 6. Subculture is generally performed when cells reach 80% confluence. Cells form sheets, indicating a high actual density. 7. Over-confluence severely impacts cell condition; cells become fragile and die after subculture. 8. The longer the culture duration, the more refractory cells are to dissociation. Terminate dissociation when most cells are visually observed to have detached. With short culture time and low density, cells can be dissociated into single cells within 3–5 minutes at 37°C. Cells cultured for more than 3 days with density above 70% are relatively refractory to dissociation, generally requiring 5–10 minutes.
Subculturing Procedure	1. Remove the culture medium from the T25 cell culture flask. 2. Add approximately 2 mL of PBS. Gently tilt the flask side to side until the PBS covers the entire bottom, then aspirate and discard the PBS. 3. Add 1 mL of 0.25% trypsin solution (containing EDTA). Gently tilt the flask side to side until the trypsin solution covers the entire bottom of the flask. 4. Incubate the cells at 37°C. Observe the cells under an inverted microscope and terminate digestion once the cells round up and detach. To avoid clumping, do not agitate the cells by tapping or shaking the flask during detachment. 5. Add 3 mL of complete culture medium to terminate digestion and disperse into a single cell suspension. 6. Collect the cell suspension and centrifuge at 1200 rpm (approximately 250 ×g) for 3 minutes. Carefully aspirate and discard the supernatant. 7. Add fresh complete culture medium, pipette gently several times to resuspend the cells, and seed them at the appropriate ratio into a new culture flask. Loosen the cap or use a vented cap for incubation.

Special Features of the Cell Line

Tumorigenic	Yes, in nude mice; forms moderately well differentiated adenocarcinoma consistent with colonic primary (grade II).
Receptor Expression	This cell line expressed heat stable enterotoxin (Sta, E. coli) and epidermal growth factor (EGF).
Genes Expressed	keratin, retinoic acid binding protein 1, retinol binding protein 2

Handling Recommendations for Cryopreserved Cells

Preparations Before Thawing

1. Prepare the recommended complete culture medium and preheat it to 37°C for 30 minutes.

2. Prepare 9 mL of complete medium within a sterile centrifuge tube to dilute the cryoprotectant present in the frozen cell suspension.
3. Use sterile gloves to protect the cryovial from contact with the water bath water, and preventing cell contamination.

Thawing Procedure

1. Retrieve the cryovial containing frozen cells from liquid nitrogen storage. Place it in sterile gloves and immediately submerge it in a 37°C water bath.
2. Thaw the cells rapidly (<1 minute) by gently swirling the vial in the 37°C water bath until only a small ice crystal remains.
3. Transfer the vial to a biosafety cabinet. Wipe the exterior of the vial with 75% ethanol before opening.
4. Transfer the thawed cells dropwise into a preheated 9 mL centrifuge tube containing complete medium.
5. Centrifuge the cell suspension at approximately 250 × g for 5 minutes.
6. After centrifugation, discard the supernatant and retain the cell pellet.
7. Gently resuspend the cells in complete growth medium, then transfer them to an appropriate culture vessel and place it in the recommended culture environment.

Notes

1. The entire recovery process should be completed as quickly as possible.
2. Select the culture vessel size based on the number of cells in the cryovial. Procell single cryovials are recommended for resuspension in T25 flasks or 10 cm dishes.
3. Minimize the time thawed cells are kept at room temperature. Cryoprotectant must be immediately diluted or removed by centrifugation.

Precautions After Receipt of Frozen Cells

1. Upon unpacking, inspect the condition of the frozen cells and dry ice, and take photographs immediately. The following after-sales service will be provided based on these photographs, including assessment of the remaining dry ice, verification that the cryovial was fully buried in dry ice, and evaluation of whether the cells thawed and refrozen during transit.
2. Upon receipt, the cells should be transferred to liquid nitrogen immediately or directly resuscitated. If liquid nitrogen is unavailable, the cells can be temporarily stored at -80°C, however, the storage period should be limited to less than two weeks whenever possible. Prolonged storage at -80°C may gradually reduce post-thaw cell viability, and the extent of this viability loss is unpredictable.
3. Ensure that the operator has sufficient knowledge and experience in cell culture, and the laboratory is equipped with essential instruments, including a biosafety cabinet, CO_2 incubator, inverted microscope, centrifuge, water bath. Carefully review the cell instruction manual to understand key cell characteristics including growth properties (adherent or suspension), morphology, basal medium requirements, serum concentration, cytokine supplementation, subcultivation ratio, medium renewal schedule.
4. After resuscitation, observe the cells under a microscope and record the cell status by taking photographs (1-3 images each at 100× and 200× magnification for 3 consecutive days). These images will serve as supporting documentation for follow-up services. In addition, a small aliquot of cells may be used to assess cell viability by automated cell counting or trypan blue staining.

Note: Cells should not be observed too frequently within the first 24 hours after resuscitation, as this may affect cell growth or adherence. Observation once per day is sufficient.

5. After sufficient expansion, the cells should be cryopreserved as soon as possible to ensure availability of backup stocks for future experiments or in case of unexpected experimental failure. Cells in the logarithmic growth phase should be selected for cryopreservation. After cryopreservation, it is recommended to resuscitate one vial to verify post-thaw viability and confirm successful cryopreservation.

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