Cell Lines Used for Research Only

D283 Med

Cat. No. : CL-0271

Origin and General Characteristics

Cell Name	D283 Med
Organism	Human
Age	6Y
Sex	Male
Tissue	Cerebellum; derived from metastatic site: ascites
Morphology	Epithelial-like
Growth Properties	Semi-adherent and semi-suspension
Background	The D283 Med cell line was established in 1985 by Friedman et al. from the ascitic fluid of a 6-year-old Caucasian male with medulloblastoma and peritoneal metastasis.
Biosafety Level	BSL-1

Culture Conditions and Handling

Complete Medium	MEM, with NEAA[PM150410]+10% Nutrient+1% Supplement
Subcultivation Ratio	1:2-1:4
Medium Renewal	2 to 3 times per week
Dissociation Duration	3-4 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.
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. This cell line proliferates slowly and has a long recovery period after cryopreservation. Medium changes should be performed every 4 days. If predominantly adherent growth is desired during culture, follow this recovery protocol: When adherent density is below 60%, collect floating cells during medium changes and return them to the original flask. When adherent density exceeds 60%, floating cells may be discarded during medium changes. Subculture when adherent density reaches 80%.

Subculturing Procedure	1. This cell line displays mixed adherent and suspended phenotypes. Although suspended cells remain viable, their density is low and they may be aspirated and discarded directly. 2. Rinse the adherent cells with PBS, then add 1–2 mL of 0.25% Trypsin-EDTA to the culture flask. Incubate at 37°C. Once the cells round up and detach from the vessel surface, add 4–6 mL of complete medium to terminate digestion. Gently pipette to generate a single-cell suspension, then harvest cells by centrifugation. 3. Seed the cells into fresh culture vessels at the recommended split ratio.

Special Features of the Cell Line

Tumorigenic	Yes, in nude mice(The cells form serially transplantable intercranial and subcutaneous tumors.) Yes, in soft agar.
Genes Expressed	The cells produce tumors in nude mice and the resulting tumors are glutamine synthetase positive; neuron specific enolase positive; glial fibrillary acidic proteins negative; S100 (S-100) protein negative; The cells express elevated levels of four biochemical markers of SCLC (neuron specific enolase; the brain isoenzyme of creatine kinase; L-DOPA decarboxylase; bombesin-like immunoreactivity)

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.