BT-20 (3D) Cell Line

Cat. No. : CL-0324-3D

Origin and General Characteristics

Cell Name	BT-20 (3D)
Organism	Human
Tissue	Breast
Morphology	Tight 3D spheres
Growth Properties	Suspension spheroids
Background	BT-20 (3D) tumorspheres are cancer stem cell-enriched spheroids obtained from BT-20 cells by enrichment and culture with specialized medium.
Biosafety Level	BSL-1

Culture Conditions and Handling

Complete Medium	Complete culture medium for BT-20 (3D)[CM-0324-3D]
Subcultivation Ratio	0.5×10⁵ cells/mL
Medium Renewal	2 to 3 times per week
Dissociation Duration	3-5 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. When cultured in non-low-adherence vessels, tumor spheres may grow with partial surface attachment.
Subculturing Procedure	1. Transfer the tumor spheres to a 15 mL centrifuge tube, centrifuge, and discard the supernatant. 2. Add approximately 1 mL of Accutase Cell Detachment Solution (Cat. No.: PB180201), and gently invert the tube to ensure the spheroids are fully submerged. 3. Incubate the tube at 37°C for 3–5 min, gently pipette the suspension up and down several times and examine under a microscope to confirm that the spheroids have dissociated into single cells or small clusters. Avoid excessive pipetting. 4. Collect the cell suspension, centrifuge at 1200 rpm for 3 min, then aspirate and discard the supernatant. 5. Resuspend the cell pellet with BT-20 (3D Spheroid-Specific) Cell Complete Medium, gently pipette to prepare a homogeneous cell suspension, seed into new culture vessels at the recommended density (e.g., 5 × 10⁴ cells/mL) and adjust to the appropriate final

culture volume with fresh medium. Loosen the cap or use a vented cap.

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 2 mL centrifuge tube containing complete medium.
5. Centrifuge the cell suspension at approximately 250 ×g for 3 minutes.
6. Aspirate and discard the supernatant, then resuspend the cells in 1 mL of BT-20(3D) complete medium.
7. Seed cells at a density of 50,000 cells/mL into 125 mL shake flasks (or low-adherence culture plates / low-adherence T25 flasks), add 10–20 mL of BT-20 (3D) complete medium, mix thoroughly, and incubate at 37°C with 5% CO_2.

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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