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## COG-E-352 Cell Line Data Sheet

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<b>Cell Line Name:</b>	<b>COG-E-352</b>
<b>Disease:</b>	Primitive neuroectodermal tumor (PNET)
<b>Phase of Therapy:</b>	Post-Chemotherapy (Progressive Disease)
<b>Prior Therapy:</b>	Vincristine/adriamycin/cyclophosphamide alternating with ifosfamide/etoposide followed by high dose carboplatin/ifosfamide/etoposide, followed by bone marrow transplant
<b>Disease Stage:</b>	
<b>Source of Culture:</b>	Blood (post-mortem)
<b>Primary Tumor Site:</b>	Fibula
<b>Date Established:</b>	February 2007
<b>EWS/FLI1 Status:</b>	ERG
<b>p53 Status:</b>	Non-functional
<b>Karyotype:</b>	
<b>Modal No:</b>	
<b>IC90 (DIMSCAN*):</b>	Pending
<b>Gender:</b>	Male
<b>Age:</b>	17 years
<b>Race:</b>	
<b>STR Profile:</b>	On file at <a href="http://strdb.cogcell.com">http://strdb.cogcell.com</a>
<b>Media Formulation:</b>	Cells are grown in a base medium of Iscove's Modified Dulbecco's Medium plus the following supplements (to a final concentration): 20% Fetal Bovine Serum, 4mM L-Glutamine, 1X ITS (5 µg/mL insulin, 5 µg/mL transferrin, 5 ng/mL selenous acid)
<b>Growth Conditions:</b>	37.0°C, 5% CO <sub>2</sub> , 20% O <sub>2</sub>
<b>Subculturing:</b>	Next page or Protocols section at <a href="http://www.cogcell.org/protocols.php">http://www.cogcell.org/protocols.php</a>
<b>Doubling Time:</b>	28 hours
<b>Morphology:</b>	Round cells and flat, epithelial-like cells
<b>Growth Properties:</b>	Suspended, grow mostly in large clumps, small population of attached cells
<b>Notes:</b>	COGcell.org has cell line available from this same patient which was established in hypoxic (5% O <sub>2</sub> ) conditions - (COG-E-352h)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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### GROWING CELLS FROM FROZEN CULTURES

To ensure that cells are recovered at the highest viability possible, immediately process cells and place them in culture. If the cells cannot be processed immediately, store the frozen vial in liquid nitrogen vapor (-196°C) upon receipt. Storing cells at -80°C will decrease the viability of the cells.

1. Thaw the vial in a water bath (37°C) for approximately two minutes. To prevent contamination, place the vial in a small ziplock bag or be sure to keep the O-ring and cap out of the water. Thawing should take no longer than 2-3 minutes.
2. While the vial is thawing, place 9 mL of the recommended complete cell culture medium in a centrifuge tube.
3. After the vial has thawed, record the label contents prior to processing. Spray the outside of the vial with 70% ethanol. Remove the contents of the vial and place it in the centrifuge tube containing the complete medium. Spin the tube at 500 x g for 5-7 minutes.
4. After spinning, decant the supernatant and resuspend the pellet in 13 mL of the complete medium. Transfer the cell suspension to a 75 cm<sup>2</sup> culture flask. Incubate the cells at 37°C in 5% CO<sub>2</sub> and room oxygen tension (~20-21% O<sub>2</sub>).

### MEDIUM RENEWAL

Protocols for medium renewal and subculturing are for a 75 cm<sup>2</sup> flask. If using different sized flasks or other cell culture vessels, adjust volumes accordingly. Cells must be renewed with new medium or subcultured at least every 3-5 days. If cells are not ≥80% confluent after 3-5 days, replace the medium with fresh medium and incubate until they are ready to be subcultured.

1. Remove supernatant from flask and place it in a 15 mL centrifuge tube. Spin the medium at 500 x g for 5 to 7 minutes.
2. After spinning, resuspend the pellet in 13 mL of complete medium and place the suspension back into the 75 cm<sup>2</sup> flask. Be sure to slowly place the medium back into the flask as to not disturb attached or loosely attached cells.

### SUBCULTURING CELLS

1. Remove supernatant from flask and place it in a 50 mL centrifuge tube. Add 10-13 mL of Puck's EDTA (see formulation below) to the flask. Be sure the solution is completely covering the plastic surface. Place the flask in the incubator for 10-15 minutes.
2. After incubation, ensure attached cells have detached by gently hitting or shaking the flask. If cells are difficult to detach, incubate flask for 5-10 minutes longer or use a cell scraper to remove remaining cells.
3. When the cells have detached, remove cell suspension and combine with the supernatant medium previously placed in the 50 mL centrifuge tube. (If a cell count is required, cells can be counted after this step). Spin the tube at 500 x g for 5-7 minutes.
4. Decant the supernatant and resuspend cells in fresh complete medium and add the appropriate aliquots of the cell suspension to new culture vessels. Seed cells at a ratio of 1:3 to 1:6.
5. Incubate the cells at 37°C in 5% CO<sub>2</sub> and 20% O<sub>2</sub>.

### PRESERVING CELLS

Cells should be frozen in a mixture of 50% FBS, 7.5% DMSO, and base medium (with no supplements) and stored in liquid nitrogen vapor.

### PUCK'S EDTA

Puck's EDTA can be made using the following cell culture grade chemicals at defined concentrations: 140 mM NaCl, 5 mM KCl, 5.5 mM Glucose, 4 mM NaHCO<sub>3</sub>, 13 μM Phenol Red, 0.8 mM EDTA, and 9 mM HEPES.

The solution should be sterile filtered (0.22 μm) before use. The optimal pH for the solution is a range between 7.2-7.3.

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**References:**

1. Batra S, Reynolds CP, Maurer BJ. Fenretinide cytotoxicity for Ewing's sarcoma (ES) and primitive neuroectodermal Tumor (PNET) cell lines is decreased by hypoxia and synergistically enhanced by ceramide modulators. *Cancer Research* 64: 5415-5424, 2004. PubMed ID: [15289350](https://pubmed.ncbi.nlm.nih.gov/15289350/)

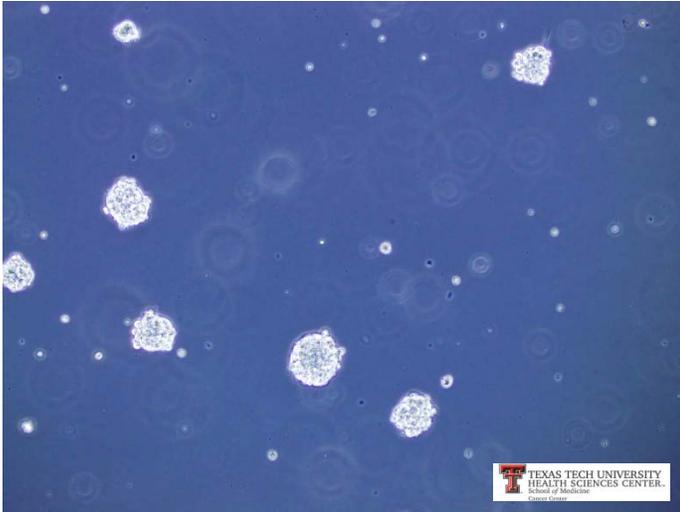
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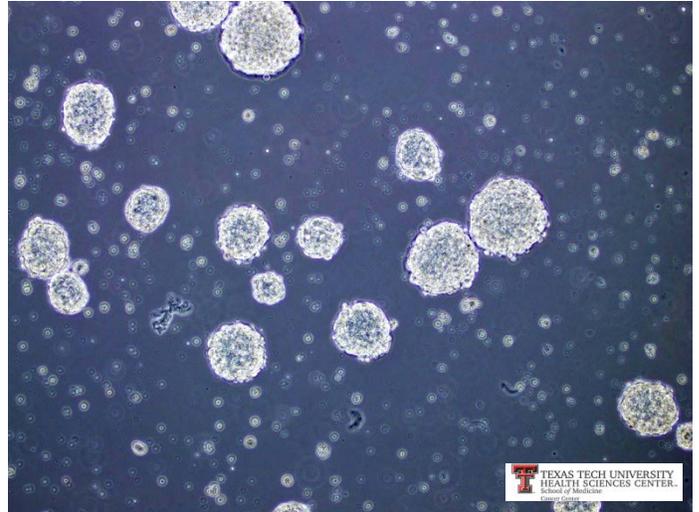
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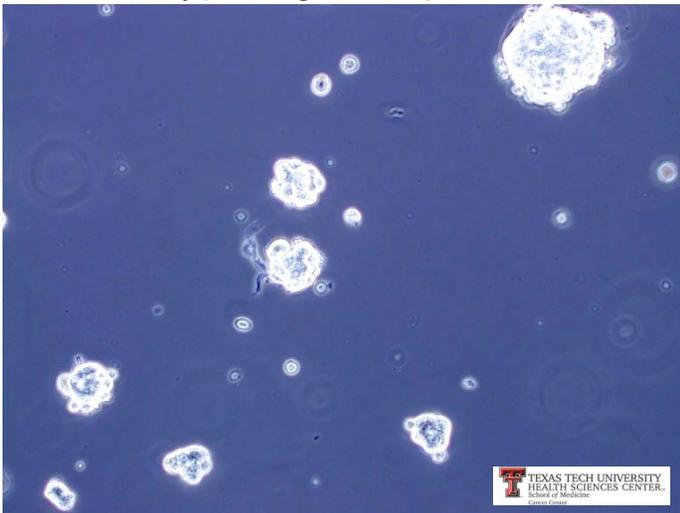
Low confluency (10x magnification)



High confluency (10x magnification)



Low confluency (20x magnification)



High confluency (20x magnification)

