

**TT Cells | 305027**

<b>Description</b>	TT cells continuously produce high levels of calcitonin and CEA. Immunoreactive calcitonin was found to be produced in cell culture at levels of 3900 pg/million cells and 7700 pg/million cells 24 and 72 hours respectively, after a medium change. CEA was found to accumulate to greater than 27 ng/million cells over a 72 hours period. Chromosomal analysis of the cell line and tumors induced in nude mice reveal an aneuploid human karyotype with several marker chromosomes. The initial characterization studies of the TT cell line were conducted using early passage TT cells cultivated in RPMI 1640 medium supplemented with 15% fetal bovine serum and 1mM L-glutamine. It is not known if the neuropeptides reported to be produced by this cell line when it was grown in RPMI 1640 medium are also produced by the cells when they are cultured in Ham's F-12K medium. Chromosomal analysis of the cell line and tumors induced in nude mice reveal an aneuploid human karyotype with several marker chromosomes.
<b>Organism</b>	Human
<b>Tissue</b>	Thyroid, medulla
<b>Disease</b>	Hereditary thyroid gland medullary carcinoma, Multiple endocrine neoplasia type 2
<b>Metastatic site</b>	Not applicable (primary hereditary medullary thyroid carcinoma; no documented distant metastasis)
<b>Applications</b>	Medullary thyroid carcinoma research; neuroendocrine tumor biology; calcitonin secretion studies; MEN2 biology; RET proto-oncogene pathway analysis; drug sensitivity (cabozantinib, vandetanib, everolimus); neuroendocrine biomarker research; CEA assay development
<b>Synonyms</b>	MTC-TT
<b>Age</b>	77 years
<b>Gender</b>	Female
<b>Ethnicity</b>	European
<b>Morphology</b>	Epithelial-like
<b>Cell type</b>	Neuroendocrine cells (C cells / parafollicular cells)
<b>Growth properties</b>	Adherent

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<b>Citation</b>	TT (Cytion catalog number 305027)
<b>Biosafety level</b>	1
<b>NCBI_TaxID</b>	9606
<b>CellosaurusAccession</b>	CVCL_1774
<b>GMO Status</b>	No genetic modification; wildtype hereditary medullary thyroid carcinoma cell line
<b>Protein expression</b>	Calcitonin, Carcinoembryonic Antigen(CEA)
<b>Tumorigenic</b>	Yes
<b>Culture Medium</b>	Ham's F12K Medium, w: 2.0 mM L-Glutamine, w: 2.0 mM Sodium pyruvate, w: 2.5 g/L NaHCO3 (Cytion article number 820608a)
<b>Supplements</b>	Supplement the medium with 10% FBS, 1% NEAA and 1mM Sodiumpyruvat
<b>Dissociation Reagent</b>	Accutase
<b>Doubling time</b>	approx. 36 to 48 hours
<b>Subculturing</b>	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
<b>Split ratio</b>	1 to 3
<b>Seeding density</b>	1 to 3 × 10 <sup>4</sup> cells/cm <sup>2</sup>

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**Fluid renewal** 2 to 3 times per week

**Post-Thaw Recovery** After thawing, plate the cells at  $5 \times 10^4$  cells/cm<sup>2</sup> and allow at least 24 hours for adherence before the first medium change. Note: Calcitonin production may require 24–72 hours post-thaw before reaching stable secretion levels.

**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

**Incubation Atmosphere** 37°C, 5% CO<sub>2</sub>, humidified atmosphere.

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#### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

#### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

#### Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.