**Product sheet**

# HT55 Cells | 305018

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| **Description** | HT55 is a human rectal adenocarcinoma cell line established from the primary rectal tumor of a 54-year-old Caucasian female. The line grows as an adherent monolayer with epithelial-like morphology and is registered in Cellosaurus as CVCL_1294. HT55 carries multiple heterozygous and homozygous loss-of-function mutations in the *APC* (adenomatous polyposis coli) tumor suppressor gene, including p.Gln1131Ter, p.Gln1303Ter, p.Arg1463Ser, p.Asn581Tyr, and p.Lys241Ter (all heterozygous), as well as a homozygous p.Arg213Leu substitution. This mutational profile renders HT55 a biologically relevant model of APC-driven colorectal carcinogenesis, reflecting the canonical WNT pathway dysregulation that drives the majority of human colorectal cancers.<br><br>HT55 is widely used in colorectal cancer research for studies of WNT/β-catenin signalling, APC tumor suppressor function, drug sensitivity and resistance, and preclinical evaluation of targeted agents against components of the WNT pathway. The line is suitable for in vitro pharmacological assays and xenograft studies in immunocompromised mouse models, providing an adherent, tractable in vitro platform for mechanistic and translational colorectal cancer research. The presence of multiple APC truncation mutations makes HT55 representative of the microsatellite-stable, chromosomally unstable colorectal cancer subtype commonly driven by APC loss-of-function.<br><br>HT55 is maintained in EMEM (MEM Eagle, w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO3, w: EBSS; Cytion article number 820100a) supplemented with 10% FBS at 37°C in a humidified atmosphere with 5% $CO_2$. Cells are subcultured using Accutase when approximately 80–90% confluent, with a recommended split ratio of 1:3 to 1:5 and a seeding density of 1–3 × $10^4$ cells/$cm^2$. Medium is renewed every 2–3 days. The doubling time is approximately 28 hours. |
| **Organism** | Human |
| **Tissue** | Rectum |
| **Disease** | Adenocarcinoma |
| **Applications** | Colorectal cancer research; WNT/APC pathway studies; drug sensitivity and resistance testing; preclinical oncology; xenograft models; targeted therapy evaluation |
| **Synonyms** | HT55 |

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| **Age** | 54 years |
| **Gender** | Female |
| **Ethnicity** | Caucasian |

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| **Morphology** | Epithelial-like |
| **Cell type** | Epithelial cells |
| **Growth properties** | Adherent |

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| **Citation** | HT55 (Cytion catalog number 305018) |
| **Biosafety level** | 1 |
| **NCBI_TaxID** | 9606 |
| **CellosaurusAccession** | CVCL_1294 |

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| **Mutational profile** | Mutation: p.Gln1131Ter, Heterozygous; Mutation: p.Gln1303Ter, Heterozygous; Mutation: p.Arg1463Ser, Heterozygous; Mutation: p.Asn581Tyr, Heterozygous; Mutation: p.Lys241Ter, Heterozygous; Mutation: p.Arg213Leu, Homozygous |

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| **Culture Medium** | EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO3, w: EBSS (Cytion article number 820100a) |
| **Supplements** | Supplement the medium with 10% FBS |
| **Dissociation Reagent** | Accutase |
| **Doubling time** | 28 hours |
| **Subculturing** | 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 300×g for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium. |

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| **Split ratio** | 1 to 5 |
| **Seeding density** | 1 to 3 x $10^4$ cells/$cm^2$ |
| **Fluid renewal** | Every 2 to 3 days |
| **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.

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| **Incubation Atmosphere** | 37°C, 5% $CO_2$, 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 °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 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen. |