Product sheet

CT26.CL25 Cells | 305353

Description	The CT26.CL25 cell line is a murine colon carcinoma model derived from the parental CT26 cell line, which is a chemically induced, undifferentiated colon carcinoma originating from BALB/c mice. CT26.CL25 has been genetically modified to express the β-galactosidase (β-gal) protein, making it an excellent model for studying tumor immunology and immunotherapy, particularly in the context of tumor-associated antigens (TAAs). This modification allows for specific immunological studies targeting β-gal as a neoantigen, facilitating research into the mechanisms of tumor immune evasion and the development of cancer vaccines or adoptive cell therapies. CT26.CL25 has been employed in preclinical models to investigate immune responses and the efficacy of immunotherapies, such as the use of dendritic cells (DCs) loaded with tumor-associated antigens. Studies have shown that immunization strategies using DCs pulsed with peptides derived from retroviral antigens, like gp70, can elicit robust anti-tumor immune responses. In experimental models, the activation of CD8+ cytotoxic T lymphocytes (CTLs) specific for gp70 was observed, demonstrating the cell line's utility in testing immunotherapeutic approaches. However, the immunization with such peptide-loaded DCs has shown limitations, particularly in treating established metastases, highlighting the challenges in translating prophylactic immune responses into therapeutic efficacy. Additionally, CT26.CL25 is often used in research to test the efficacy of combined immunotherapy approaches, such as the use of immune checkpoint inhibitors or cancer vaccines. For instance, studies have evaluated the impact of metronomic chemotherapy combined with immune checkpoint inhibitors, where the induction of immunogenic cell death (ICD) in CT26.CL25 has been crucial for enhancing the anti-tumor immune response. These investigations have demonstrated that targeting immune checkpoints can synergize with chemotherapy to increase tumor rejection rates and establish long-term immunological memory.
Organism	Mouse
Tissue	Colon
Disease	Adenocarcinoma
Synonyms	CT26-clone 25

Breed/Subspecies	BALB/c
Age	Unspecified
Gender	Female
Morphology	Fibroblast

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Growth properties	Adherent

Citation	CT26.CL25 (Cytion catalog number 305353)
Biosafety level	1
NCBI_TaxID	10090
CellosaurusAccession	CVCL_7255
GMO Status	GMO-S1: This murine colon carcinoma cell line (CT26.CL25) contains a retroviral vector encoding lacZ and Tn5-neo, enabling β-galactosidase expression and neomycin resistance. The construct is stably integrated into CT26 cells. This classification applies only within Germany and may differ elsewhere.

Antigen expression	H-2d
Tumorigenic	Yes, in BALB/c mice
Products	Genes expressed: beta galactosidase (beta-gal), H-2D
Mutational profile	Gene deletion: Cdkn2a, homozygous; Mutation: Kras, p.Gly12Asp (c.35G>A), homozygous

Culture Medium	RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO3 (Cytion article number 820700a)
Supplements	Supplement the medium with 10% FBS, 1% NEAA, 0.4 mg/mL G418, add 2.5 g/L glucose and 10 mM HEPES
Dissociation Reagent	Accutase

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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 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
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_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.
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.