CT26.CL25

305353

GENERAL INFORMATION

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.

Tissue	Colon
Disease	Adenocarcinoma

Synonyms

- CT26-clone 25

CLASSIFICATION

Organism	Mouse
Sex	Female
Growth properties	Adherent
GMO status	GMO-S1

GMO status details

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.

Biosafety level	1

CHARACTERISTICS

Morphology	Fibroblast
Tumourigenic	Yes, in BALB/c mice

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IDENTIFIERS

Citation

CT26.CL25 (Cytion catalog number 305353)

NCBI TaxID	10090
Cellosaurus	CVCL_7255

EXPRESSION AND SUSCEPTIBILITY

Antigen expression

H-2d

Mutational profile

Gene deletion: Cdkn2a, homozygous; Mutation: Kras, p.Gly12Asp (c.35G>A), homozygous

Products

Genes expressed: beta galactosidase (beta-gal), H-2D

HANDLING AND CULTURE

Culture medium

RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO3 (Cytion article number 820700a)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

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

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.

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

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.

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.

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.

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.

DONOR

Breed / subspecies	BALB/c
Donor age	Unspecified

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Material Transfer Agreement required	Yes