Data Sheet

CT-2A-Luc Mouse Glioma Cell Line

Immortalized Cell Line

SCC195

Pack Size > 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Glioblastomas are among the most aggressive forms of cancer, associated with low treatment efficacy and poor survival. Recurring glioblastomas are often resistant to first-line chemotherapies.[1] There is much interest in studying drug-resistant forms of glioblastomas in the effort to develop effective therapies.

The CT-2A luciferase cell line is derived from a sub-cutaneous, non-metastatic murine glioma (astrocytoma) and is stably transduced with a firefly luciferase-EGFP reporter. CT-2A luciferase cells are marked by high levels of complex gangliosides and low distribution of the anti-angiogenic ganglioside GM3, as well as deficiency in the tumor suppressor PTEN/TSC2, a characteristic present in up to 70% of human high-grade glioma cell lines.[2,3] CT-2A tumors are wild-type for p53 and recapitulate several features of human high-grade glioma, including high mitotic index and cell density, nuclear polymorphism, hemorrhage, pseudo palisading necrosis, and microvascular proliferation. These attributes have contributed to use of the CT-2A luciferase cell line a valuable model for therapeutic research on brain malignancies.

Source

The parent CT-2A cell line was generated from a malignant astrocytoma formed via implantation of the carcinogen 20-methylcholanthrene in the cerebrum of a C57BL/6J mouse.[4] The tumor was maintained through serial intracranial transplants prior to cell line isolation. The CT-2A luciferase cell line was clonally derived from CT-2A cells transduced with a lentiviral vector harboring a firefly luciferase (Fluc)-IRES-GFP cassette under control of the CMV promoter.[5] The CT-2A-Luc cell line does not retain GFP expression but retains luciferase expression.

Short Tandem Repeat

M1-1: 17	M1-2: 19	M3-2: 14	M4-2: 20.3	M5-5: 17	M6-4: 18	M6-7: 17
M7-1: 26.2	M8-1: 16	M11-2: 16	M12-1: 17	M13-1: 17	M15-3: 22.3	M17-2: 15
M18-3: 16	M19-2: 13	MX-1: 28	M2-1: 16			

Quality Control Testing

- CT-2A-Luc cells are verified to be of mouse origin and negative for human, rat, non-human primate, Chinese hamster, and Golden Syrian hamster interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

CT-2A-Luc cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

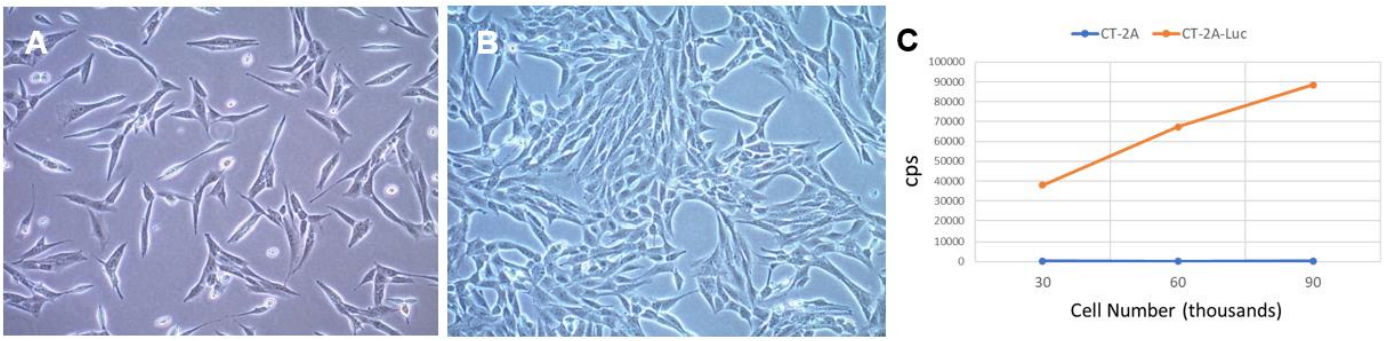

Figure 1. Bright-field image of CT-2A-Luc cells one (**A**) and two (**B**) days after thaw in a T75 flask. (**C**) Luciferase signal (cps) increases with cell number in CT-2A-Luc cells. Parental CT-2A cells do not show luciferase signal.

Protocols

Thawing Cells

Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

1. Cells are thawed and expanded in DMEM High Glucose (D6429), 10% FBS (ES-009-B), and 1X Penicillin-Streptomycin Solution (TMS-AB2-C) (optional).
2. Remove the vial of frozen CT-2A cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of CT-2A Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of CT-2A Expansion Medium.

10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of CT-2A cells.
2. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 mL of Accutase® or trypsin-EDTA solution and incubate in a 37 °C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 5-7 mL of CT-2A Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of CT-2A Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

CT-2A-Luc cells may be frozen in CT-2A-Luc Expansion Medium supplemented with 10% FBS and 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Weller M, Cloughesy T, Perry J R, and Wick W (2013). Standards of care for treatment of recurrent glioblastoma- are we there yet? Neuro Oncol 15:4-27.
2. Seyfried T and Mukherjee P. (2010) Ganglioside GM3 is antiangiogenic in malignant brain cancer. J Oncol 2010:961243 doi.10.1155/2010/961243.
3. Cotterchio M and Seyfried T (1994). Serum gangliosides in mice with metastatic and non-metastatic brain tumors. J Lipid Res 35:10-14.
4. Zimmerman H M and Arnold H. (1941). Experimental brain tumors. I. Tumors produced with methylcholanthrene. Cancer Res 1(12):919-938.
5. Huysentruyt L, Mukherjee P, Banerjee D, Shelton L, and Seyfried T (2008). Metastatic cancer cells with macrophage properties: Evidence from a new murine tumor model. Int J Cancer 123:73-84.

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