CT-2A Mouse Glioma Cell Line

Cancer Cell Line

Cat. # SCC194

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.

Data Sheet

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 cell line is derived from a sub-cutaneous, non-metastatic murine glioma (astrocytoma). The originating tumor was classified as poorly differentiated with high vascularity and malignancy.[2] CT-2A 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.[3,4] 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, pseudopalisading necrosis, and microvascular proliferation.[5,6] These attributes have contributed to use of the CT-2A cell line is a valuable model for therapeutic research on brain malignancies.

Source

CT-2A was generated from a malignant astrocytoma formed via implantation of the carcinogen 20-methylcholanthrene in the cerebrum of a C57BL/6J mouse.[7] The tumor was maintained through serial intracranial transplants prior to cell line isolation.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for inter-species contamination from rat, chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells are negative for mycoplasma contamination.

Storage and Handling

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

Representative Data

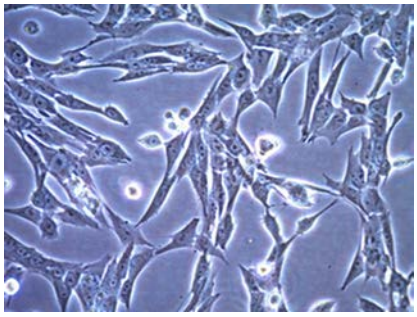

Figure 1. CT-2A cells one day after thawing in a T75 flask.

References

1. Weller M, Cloughesy T, Perry JR, and Wick W (2013). *Neuro Oncol* 15(1): 4-27.
2. Seyfried TN, el-Abbadi M, and Roy ML (1992). *Mol Chem Neuropathol* 17(2):147-167.
3. Seyfried TN, Mukherjee P (2010). *J Oncol* 2010:961243 doi.10.1155/2010/961243.
4. Cotterchio M, Seyfried TN (1994). *J Lipid Res* 35(1): 10-14.
5. Binello E, Qadeer ZA, Kothari HP, Emdad L, Germano IM (2012). *J Cancer* 3: 166-174.
6. Martinez-Murillo R, Martinez A (2007). *Histol Histopathol* 22(12): 1309-1326.
7. Zimmerman HM and Arnold H. (1941). *Cancer Res* 1(12): 919-938.

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Protocols

Thawing Cells

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

   CT-2A Expansion Medium: Cells are thawed and expanded in DMEM High Glucose (Sigma Cat. No. D6429), 10% FBS (Cat. No. ES-009-B), and 1X Penicillin-Streptomycin Solution (Cat. No. 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 Cells

CT-2A mouse glioma cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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