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Data Sheet

# CNS-1 Rat Glioma Cell Line

Cancer Cell Line

**SCC487** 

Pack Size >1x106

Store at: liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

## Background

Glioma is one of the most common brain tumors and is highly aggressive and resistant to standard treatments. Immunotherapy is one of the more promising treatment options since the discovery of lymphatics in the central nervous system allows for penetration of the blood-brain barrier. Cellular models that both recapitulate in vivo characteristics of gliomas and are amenable to immunotherapeutic modalities are thus of great value in advancing understanding of gliomas and their potential treatment.

The CNS-1 rat glioma cell line is a well-established model that closely mimics the invasive behavior and growth characteristics of human gliomas.<sup>2</sup> CNS-1 cells are derived from an N-nitroso-N-methylurea-induced intracranial tumor of a male Lewis rat and are histocompatible with this inbred rat strain, facilitating a range of in vivo studies. Tumors derived from injection of CNS-1 cells display diffuse infiltrative patterns similar to human glioblastoma multiforme.<sup>2,3</sup> CNS-1 cells are positive for glial fibrillary acid protein (GFAP), retinoic acid receptor a, and neural cell adhesion molecule NCAM-140.2,4 CNS-1 cells exhibit multiple chromosome loss and rearrangements<sup>2</sup> reflecting genetic instability common in many human gliomas. CNS-1 rat glioma cells are a highly relevant model with wide applications for glioma immunotherapies.

#### Source

CNS-1 cell line originated from an N-nitroso-N-methylurea-induced intracranial tumor of a male Lewis rat.<sup>1</sup>

## **Quality Control Testing**

- Each vial contains ≥ 1X10<sup>6</sup> 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 rat origin and negative for inter-species contamination from human, mouse, Chinese hamster, Golden Syrian hamster, 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

CNS-1 rat glioma cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.



### Representative Data

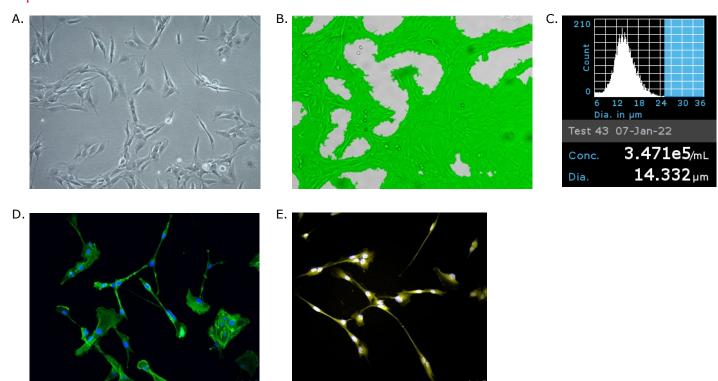


Figure 1. Bright-field image of CNS-1 cells one day after thaw in a T75 flask (A). Cell confluency (80%) was assessed throughout the culture using MilliCell® Digital Cell Imager (B, Cat. No. MDCI 10000). Cell counting was performed using Scepter 3.0 handheld automated cell counter using 60  $\mu$ m sensor tips (C, PHCC360KIT). CNS-1 cells express actin (D, Cat. No. 94072) and the glial marker, GFAP (E, Cat. No. AB5541).

### **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.
  - Cells are thawed and expanded in CNS-1 Expansion Medium comprised of RPMI medium (Cat. No. R0883) with 10% FBS (Cat. No. ES-009-B), 2 mM L-Glutamine (Cat. No. TMS-002-C), and 1X penicillin/streptomycin (Cat. No. TMS-AB2-C, optional).
- 2. Remove the vial of frozen CNS-1 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 50 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 CNS-1 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.

- 7. 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.
- 8. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
- 9. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
- 10. Resuspend the cells in 15 mL of CNS-1 Expansion Medium.
- 11. Transfer the cell mixture to a T75 tissue culture flask.
- 12. Incubate the cells at 37 °C in a humidified incubator with 5% CO<sub>2</sub>.

#### Subculturing Cells

- 1. CNS-1 cells should be passaged at ~80-85% confluency. Do not allow the cells to grow over 85% confluency.
- 2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of CNS-1 cells. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
- 3. Apply 5 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 8 mL of CNS-1 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 CNS-1 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 or a Scepter™ 3.0 handheld automated cell counter using 60 µm sensor tips.
- 11. Plate the cells to the desired density. Typical split ratio is 1:6–1:10. The medium should be replaced every other day. Cells proliferate rapidly.

#### Cryopreservation of Cells

CNS-1 rat glioma cells may be frozen in CNS-1 Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

### References

- 1. Cancer Lett 2020, 476: 1-12.
- 2. J Neurooncol 1994, 22(3): 191-200.
- 3. J Neurooncol 2009, 94(3): 299-312.
- 4. Cancer Res 1998, 58(9): 2020-2028.

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