cyagen | OriCell

User Manual

OriCell™ 9L/lacZ Rat Gliosarcoma Cell Line

Catalog No. R8-0401

We help you discover life

Introduction

Rat gliosarcoma cell line (9L/lacZ) was developed in 1989 from the 9L cell line, which was originally induced from rat brain tumors (gliomas). The 9L cells were infected with a replication-defective retroviral vector carrying the E. coli lacZ gene encoding β-galactosidase and the Tn5 neomycin resistance gene (BAG), conferring G418 resistance. Cells were cultured in the presence of G418 for 14 days, followed by cloning and screening for β-galactosidase expression, resulting in the successful establishment of the 9L/lacZ cell line.

OriCell ™ 9L/lacZ Rat Gliosarcoma Cell Line is widely used in studies of glioma biology, tumor microenvironment and its interaction with the immune system, as well as drug development and therapeutic research.

Note: **This product is intended for research use only and is not for clinical treatment or any other purposes.**

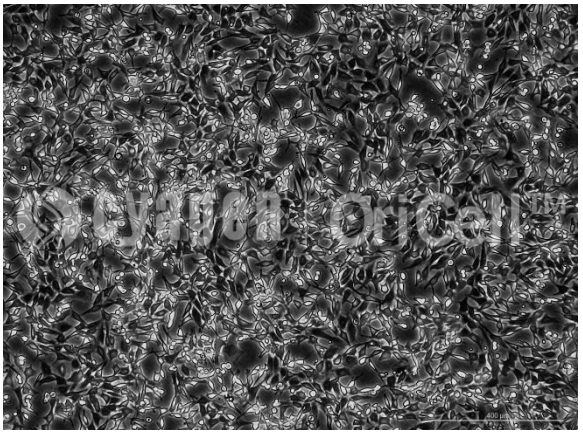
When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences."

Product Information

Name	OriCell™ 9L/lacZ Rat Gliosarcoma Cell Line
Alternative Name	9L/LacZ
Catalog Number	R8-0401
Amount of Cells	1×10^6 cells/vial
Tissue Origin	Rat Brain
Cell Characteristics	Adherent Growth; Fibroblast-like
Culture Conditions	95% air; 5%CO_2; 37°C
Culture Medium	DMEM + 10% FBS
Doubling Time	48~72 h
Biosafety Level	1
Storage at	Liquid Nitrogen (-196°C)
Precautions	——

Note: This product is manufactured under strict aseptic conditions. You may choose to add antibiotics during subsequent culturing based on your specific needs.

The Morphology of OriCell™ 9L/lacZ Rat Gliosarcoma Cell Line



QC

- Tested for bacteria, fungi, mycoplasma, and endotoxins.
- Tested for cell viability and recovery.
- Confirmed by short tandem repeat (STR) analysis.

Please refer to "COA" for details.

General Handing Principles

1. Strict sterile environment. Ensure the cleanliness of the entire laboratory, laminar flow hood, and incubator.
2. Follow standardized operating procedures. Adhere to the instructions described in the product manual, strictly control variables, and conduct appropriate control experiments.
3. Use appropriate and high-quality consumables and reagents. This product requires culture vessels suitable for adherent cell growth, and their reuse is not recommended. The reagents used must be validated for reliability, suitable for cell growth, and exhibit minimal batch-to-batch variation.

Note: **The cryopreservation solution of this product contains DMSO, which may pose potential risks. Please handle it with care.**

Abbreviation	Name	Cat. No.
FBS	Fetal Bovine Serum	See official website
BCS	Bovine Calf Serum	SBCST-01001
Glu	Glutamine	SGLU-10201
SP	Sodium Pyruvate	SCSP-10301
Dex	Dexamethasone	SDEX-10401

NBCS	Newborn Calf Serum	NCSST-01001
HS	Horse Serum	SCHST-01001
NEAA	Non Essential Amino Acid	NEAA-10201
β-mer	β-mercaptoethanol	BMER-10301
P/S	Penicillin- Streptomycin	ATPS-10001
ITS	Insulin, Transferrin, Selenite	ITSS-10201

Thawing and Establishing of Cells

Materials Required

- OriCell™ 9L/lacZ Rat Gliosarcoma Cell Line (Cat. No.: R8-0401)
- OriCell™ Complete Medium For 9L/lacZ Cell Line (Cat. No.: CMR8-0401)

Steps

Note: **If the received cells are to be thawed within 24 hours, they can be stored in an ultra-low temperature freezer at -80°C. For storage longer than 24 hours, please keep them in liquid nitrogen. Before thawing, transfer the cells from liquid nitrogen to -80°C and hold them there for 10 minutes to allow any residual liquid nitrogen in the tube to evaporate.**

1. Preheat the water bath at 37°C.
2. Warm the complete medium to 37°C.
3. Add at least 5 mL of complete medium to a 15 mL centrifuge tube for subsequent use.
4. Remove the cryovial containing cells from the −80°C freezer, immerse it in the 37°C water bath, and gently and quickly swirl to thaw the cryopreservation solution.

   Note:

   (1) Gently shake the cryovial during thawing to ensure rapid and uniform thawing.

   (2) Avoid submerging the cap in water to prevent contamination.

(3) Stop thawing in the water bath when only a single ice crystal (approximately 2 mm in diameter) remains, then continue gently shaking the vial until it fully melts.

5. Wipe the outer surface of the cryotube with 75% ethanol.
6. In a biosafety cabinet, open the cryovial and transfer the cell suspension to the prepared centrifuge tube using a Pasteur pipette.
7. Rinse the cryovial once with 1 mL of complete medium to collect residual cells and minimize loss.
8. Centrifuge the cell suspension at 250×g for 4 minutes.
9. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium, gently resuspend the cell pellet by pipetting up and down to mix thoroughly.
10. Inoculate the cells into a T25 flask or culture vessel with an equivalent growth surface area. Add sufficient complete medium so that the total volume in a T25 flask is no less than 5 mL.
11. Gently swirl the flask to evenly distribute the cells, then incubate in a CO_2 incubator at 37°C with 5% CO_2 and saturated humidity.

    Note: **Do not disturb or observe the cells within the first 2 hours after seeding, as this may affect cell adhesion, resulting in poor morphology, clumping, or uneven attachment.**

12. The day after recovery, observe the cell status and either replace the medium with fresh complete medium or passage the cells as necessary.

    Note: **If an excessive number of floating cells or any abnormal conditions are observed, investigate promptly and contact us for assistance.**

13. Refresh the complete medium every 3 days until the cells reach approximately 95% confluence, at which point passage is required.

Passaging of Cells

Materials Required

- OriCell™ 0.25% Trypsin-0.04% EDTA Solution (Cat. No.: TEDTA-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- OriCell™ Complete Medium For 9L/lacZ Cell Line (Cat. No.: CMR8-0401)

Steps

1. Prewarm the complete medium and trypsin to 37°C.
2. Remove the medium in the culture vessel.
3. Gently wash the cells twice with PBS (approximately 3 mL for a T25 flask and 6 mL for a T75 flask). Ensure thorough washing but avoid excessive force. Then remove the PBS.
4. Add trypsin (approximately 1.5 mL for a T25 flask and 3 mL for a T75 flask), quickly spread it to ensure full coverage of the cell layer.
5. Observe the cells under a microscope. When approximately 70% to 80% of the cells have shrunk and become round, gently tap the outer wall of the culture vessel to detach the cells from the surface.
6. Immediately add complete medium (approximately 3 mL for a T25 flask and 6 mL for a T75 flask), then gently swirl the culture vessel to mix the medium and trypsin, stopping the digestion process.
7. Collect the cell suspension using a pipette, gently pipetting along the bottom of the vessel several times to ensure maximal recovery of the cells.

   Note: **Pipetting should be performed gently to avoid damaging the cells.**

8. Transfer the cell suspension to a centrifuge tube. Rinse the culture vessel once with PBS (approximately 3 mL for a T25 flask and 6 mL for a T75 flask) to collect any remaining cells and add the wash to the centrifuge tube.

9. Centrifuge all collected cell suspensions at 250 × g for 4 minutes.
10. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium and gently resuspend the cell pellet by pipetting up and down to thoroughly mix.
11. Inoculate the cells into a suitable culture vessel at a density of (2–3) × 10^4 live cells/cm^2, or adjust the seeding density based on the actual growth conditions of the cells.

    Note: **We recommend manual cell counting when conditions permit and counting efficiency is high, in order to obtain an accurate cell concentration to guide seeding. If precise counting is not feasible, subculturing at an appropriate ratio is a better alternative. Typically, 9L/lacZ cells are passaged at a ratio of 1:3 to 1:5, with cells reaching passage confluence within 96 hours. Please adjust the subculture ratio according to the actual condition of the cells.**

12. On the day after passaging, observe the cell condition. If a significant number of floating cells are present, replace the culture medium.
13. Refresh the culture medium every 3 days. When cell confluence exceeds 95%, passage or cryopreserve the cells.

Cryopreservation of Cells

Materials Required

- OriCell™ NCR Protein-Free Cryopreservation Medium For General Use (Cat. No.: NCPF-10001)
- OriCell™ NCR Cryopreservation Medium For General Use (Cat. No.: NCRC-10001)

Steps

1. Cells should be cryopreserved once they reach an appropriate density suitable for passaging.
2. For cell digestion, please refer to passaging steps 1-9 above.
3. Carefully remove the supernatant after centrifugation and gently resuspend the cells in an appropriate volume of cryopreservation medium.

4. Aliquot the cells into cryovials according to the desired cell number or proportion.

   Note: **If accurate cell counting is not feasible, we recommend aliquoting cells proportionally for freezing. Prolonged storage under non-culture conditions will significantly reduce cell viability. Keep the cells at 4°C during counting to minimize metabolic activity and preserve cell integrity.**

5. When using any of the recommended NCR cryopreservation media above, cryovials can be directly placed individually into a -80°C freezer.

   Note: **Avoid opening the freezer door during the first 4 hours of freezing, as temperature fluctuations can seriously impact cell viability.**

6. After approximately 8 hours, transfer the cryovials to liquid nitrogen for long-term storage.

   Note: **Storage at -80°C should not exceed 48 hours.**