

**Data Sheet**

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**BCRJ Code:** 0029**Cell Line:** 9L/LacZ**Species:** *Rattus norvegicus***Vulgar Name:** Rat**Tissue:** Brain**Cell Type:** Glial Cell**Morphology:** Fibroblast**Disease:** Gliosarcoma**Growth Properties:** Adherent

**Derivation:** The 9L/lacZ cell line was developed in 1989 from the 9L cell line (rat nitrosourea induced gliosarcoma cell line). 9L cells were infected with the BAG replication deficient retroviral vector carrying the E. coli lacZ gene encoding beta-gal and the Tn5 neomycin gene, which confers resistance to G418. The cells were cultured in G418 for 14 days, cloned, and evaluated for beta-gal production. 9L/lacZ produced high levels of the enzyme, and was selected for study.

**Applications:** This cell line is one of few models that permit quantitative analysis of microscopic tumor in the brain. The tumor mimics important features of human brain tumor growth and spread.

**Tumor Formation::** Yes, forms tumors in the brains of CD Fischer 344 rats

**Products:** beta galactosidase (beta-gal)

**Biosafety:** 1

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### Additional Info:

The cells constitutively express the lacZ reporter gene product, E. coli derived beta-gal, as revealed on tissue sections by histochemical stain, and single tumor cells can be identified. Lymphocytes and other responding cells can be identified by double labeling with antibodies on the same slide. The contrast between stained cells and background facilitates image analysis. The beta-gal expression is very stable, but cells may need to be re-cloned after months of growth in culture.

### Culture Medium:

Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate and fetal bovine serum to a final concentration of 10%.

### Subculturing:

Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. T-75 flasks are recommended for subculturing this product. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

### Subculturing Medium Renewal:

2 to 3 times a week

### Subculturing Subcultivation Ratio:

1:4 to 1:8

### Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO<sub>2</sub>), 5% Temperature: 37°C

### Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

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**SAFETY PRECAUTION:** It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately  $125 \times g$  for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

**NOTE:** It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

Lampson LA, et al. Interactions between leucocytes and individual brain tumor cells in the rat brain. J. Neuro-Oncol. 7: S17, 1990. Lampson LA, et al. Exploiting the lacZ reporter gene for quantitative analysis of disseminated tumor growth within the brain: use of the lacZ gene product as a tumor antigen, for evaluation of antigenic modulation, and to facilitate image analysis of tumor growth in situ. Cancer Res. 53: 176-182, 1993. PubMed: 8416743 Lampson LA, et al. Disseminating tumor cells and their interactions with leukocytes visualized in the brain. Cancer Res. 52: 1018-1025, 1992. PubMed: 1737331 Dutta T, et al. Robust ability of IFN-gamma to upregulate class II MHC antigen expression in tumor bearing rat brains. J. Neuro-Oncol. 64: 31-44, 2003. PubMed: 12952284

### Thawing Frozen Cells:

### References:

### Depositors:

DEBORA AMADO; INSTITUTO DE ENSINO E PESQUISA ALBERT EINSTEIN.

### Cellosaurus:

[CVCL 5656](#)



