

## Data Sheet

**BCRJ Code:** 0188

**Cell Line:** NCTC clone 929 [L cell, L-929, derivative of Strain L]

**Species:** Mus musculus

**Vulgar Name:** Mouse; C3H/An

**Tissue:** Subcutaneous Connective Tissue; Areolar And Adipose

**Cell Type:** Connective Tissue Fibroblast

**Morphology:** Fibroblast

**Disease:** Normal

**Growth Properties:** Adherent

**Sex:** Male

**Age/Ethnicity:** 100 Day /

**Derivation:** The parent L strain was derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H/An mouse. NCTC clone 929 (Connective tissue, mouse) Clone of strain L was derived in March, 1948. Strain L was one of the first cell strains to be established in continuous culture, and clone 929 was the first cloned strain developed. Clone 929 was established (by the capillary technique for single cell isolation) from the 95th subculture generation of the parent strain.

**Applications:** This cell line can be used for toxicity testing. This cell line is a suitable transfection host.

**Virus Susceptibility::** Vesicular stomatitis, Glasgow (Indiana) Vesicular stomatitis, Orsay (Indiana) Encephalomyocarditis virus

**Virus Resistance::** poliovirus 1, 2, 3; coxsackievirus B5; polyomavirus

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<b>Tumor Formation::</b>	Yes, in immunosuppressed mice
<b>Biosafety:</b>	1
<b>Culture Medium:</b>	Dulbecco's Modified Eagle's Medium (DMEM) with 1% non-essential amino acids, 2 mM L-glutamine, 1.0 g/L glucose and 10% of fetal bovine serum, 10%.
<b>Subculturing:</b>	<p>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. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum which 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.</p>
<b>Subculturing Medium Renewal:</b>	1 to 2 times per week
<b>Subculturing Subcultivation Ratio:</b>	1:2 to 1:8
<b>Culture Conditions:</b>	Atmosphere: air, 95%; carbon dioxide (CO <sub>2</sub> ), 5% Temperature: 37°C
<b>Cryopreservation:</b>	95% FBS + 5% DMSO (Dimethyl sulfoxide)

**Thawing Frozen Cells:**

**SAFETY PRECAUTION:** It is highly recommended that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of 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 the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. For cells that are sensitive to DMSO it is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).
5. Incubate the culture in an appropriate atmosphere and temperature (see "Culture Conditions" for this cell line).

**NOTE:** It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

**References:**

Kazazian HH Jr., et al. Restriction site polymorphism in the phosphoglycerate kinase gene on the X chromosome. Hum. Genet. 66: 217-219, 1984. PubMed: 6325324 Fisher EM, et al. Homologous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome. Cell 63: 1205-1218, 1990. PubMed: 2124517 Sanford KK, et al. The growth in vitro of single isolated tissue cells. J. Natl. Cancer Inst. 9: 229-246, 1948. Sugarman BJ, et al. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230: 943-945, 1985. PubMed: 3933111 ASTM International Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 0813-07. ASTM International Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 0895-84 (Reapproved 2006). U.S. Pharmacopeia General Chapters: <87> Biological Reactivity Tests, in vitro. Rockville, MD: U.S. Pharmacopeia; USP USP34-NF29, 2011 Westfall BB, et al. The glycogen content of cell suspensions prepared from massive tissue culture: comparison of cells derived from mouse connective tissue and mouse liver. J. Natl. Cancer Inst. 14: 655-664, 1953. PubMed: 13233820 Earle WR, et al. Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. J. Natl. Cancer Inst. 4: 165-212, 1943. Earle WR, et al. The influence of inoculum size on proliferation in tissue cultures. J. Natl. Cancer Inst. 12: 133-153, 1951. PubMed: 14874126 Sanford KK, et al. The tumor-producing capacity of strain L mouse cells after 10 years in vitro. Cancer Res. 16: 162-166, 1956. PubMed: 13293658 Westfall BB, et al. The arginase and rhodanese activities of certain cell strains after long cultivation in vitro. J. Biophys. Biochem. Cytol. 4: 567-570, 1958. PubMed: 13587550

**Depositors:** Banco de Células do Rio de Janeiro

**ATCC:** CCL-1

**Cellosaurus:** [CVCL\\_0462](#)