BCRJ Code:	0141
Cell Line:	L6
Species:	*Rattus norvegicus*
Vulgar Name:	Rat
Tissue:	Skeletal Muscle
Cell Type:	Myoblast
Morphology:	Myoblastic
Growth Properties:	Adherent
Applications:	This cell line is a suitable transfection host.
Products:	myosin
Biosafety:	1
Addtional Info:	L6 cells fuse in culture to form multinucleated myotubes and striated fibers. The extent of cell fusion declines with passage and the cells should be frozen at low passage and periodically recloned with selection for fusion competent cells.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and fetal bovine serum to a final concentration of 10%.

Subculturing:	Volumes used in this protocol are for 75 cm2 flasks; 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.
Subculturing Medium Renewal:	2 to 3 times per week
Subculturing Subcultivation Ratio:	1:20 to 1:40
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide) Growth Conditions: The myoblastic component of this line will be depleted rapidly if the cells are allowed to become confluent. no campo onde ta temperatura.

Thawing Frozen Cells:	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 × 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).
References:	Mandel JL, Pearson ML. Insulin stimulates myogenesis in a rat myoblast line. Nature 251: 618-620, 1974. PubMed: 4421831 Richler C, Yaffe D. The in vitro cultivation and differentiation capacities of myogenic cell lines. Dev. Biol. 23: 1-22, 1970. PubMed: 5481965 Yaffe D. Retention of differentiation potentialities during prolonged cultivation of myogenic cells. Proc. Natl. Acad. Sci. USA 61: 477-483, 1968. PubMed: 5245982 Osawa H, et al. Identification and characterization of basal and cyclic AMP response elements in the promoter of the rat hexokinase II gene. J. Biol. Chem. 271: 17296-17303, 1996. PubMed: 8663388 Osawa H, et al. Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. J. Biol. Chem. 271: 16690-16694, 1996. PubMed: 8663315
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