Data Sheet

BCRJ Code:	0098
Cell Line:	H9c2(2-1)
Species:	*Rattus norvegicus*
Vulgar Name:	Rat/Bd1X
Tissue:	Myocardium/Heart
Cell Type:	Myoblast
Morphology:	Myoblast
Growth Properties:	Adherent
Age/Ethinicity:	EMBRYO /
Derivation:	H9c2(2-1) is a subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue by B. Kimes and B. Brandt and exhibits many of the properties of skeletal muscle.
Products:	Myokinase; creatine phosphokinase; myosin.
Biosafety:	1
Addtional Info:	Skeletal muscle properties. Myotubes formed at confluency respond to acetylcholine. Differentiation improved by reducing serum concentration to 1%.
Culture Medium:	Dulbecco's modified Eagle's medium with 4.5 g/L glucose and 10% of fetal bovine serum.

Subculturing:	NOTE: The myoblastic population will become depleted rapidly if the cultures are allowed to become confluent. To prevent loss of myoblastic cells, cultures should be subcultured before they become confluent, and the line should be recloned periodically with selection for myoblastic cells. Volumes used in this protocol are for 75 cm2 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:	Every 2 to 3 days
Subculturing Subcultivation Ratio:	1:2 to 1:4
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

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:	Kimes BW, Brandt BL. Properties of a clonal muscle cell line from rat heart. Exp. Cell Res. 98: 367-381, 1976. PubMed: 943302 Levy AP, et al. Post-transcriptional regulation of vascular endothelial growth factor by hypoxia. J. Biol. Chem. 271: 2746-2753, 1996. PubMed: 8576250
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