

Data Sheet

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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/Ethnicity:	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
Additional 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.

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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: Is highly recommend 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 submersed 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 Oring 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 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 a 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:

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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