

Data Sheet

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BCRJ Code:	0333
Cell Line:	32D clone 3
Species:	Mus musculus
Vulgar Name:	Mouse
Tissue:	Bone Marrow
Morphology:	Lymphoblast
Growth Properties:	Mixed, Adherent And Suspension
Applications:	This cell line is a suitable transfection host.
Biosafety:	1
Additional Info:	This line is IL-3 dependent.
Culture Medium:	RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 4.5 g/L glucose, 10% of heat-inactivated fetal bovine serum and 10% mouse Interleukin-3 culture supplement.
Subculturing:	Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 2 X 10 ⁵ viable cells/mL. Maintain cell density between 2 X 10 ⁵ and 1 X 10 ⁶ viable cells/mL. Scrape off the attached cells and transfer along with the floating cells into new flasks. 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
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO ₂), 5% Temperature: 37°C

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Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

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).

Thawing Frozen Cells:**References:**

Ahmed N, Berridge MV. Regulation of glucose transport by interleukin-3 in growth factor-dependent and oncogene-transformed bone marrow-derived cell lines. *Leuk. Res.* 21: 609-618, 1997. PubMed: 9301681 Ahmed N, Berridge MV. Distinct regulation of glucose transport by interleukin-3 and oncogenes in a murine bone marrow-derived cell line. *Biochem. Pharmacol.* 57: 387-396, 1999. PubMed: 9933027

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