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BCRJ Code:	0155
Cell Line:	M3/38.1.2.8 HL.2
Species:	Rattus norvegicus Rattus norvegicus (B cell); Mus musculus (myeloma), rat (B cell); mouse (myeloma)
Vulgar Name:	Rat/Mouse
Tissue:	Spleen
Cell Type:	Hybridoma: B Lymphocyte
Morphology:	Lymphoblast
Growth Properties:	Suspension
Applications:	Mac-2 and Mac-3 are present on 69% of macrophages and 0% to 2% of thymocytes
Products:	immunoglobulin; monoclonal antibody; against mouse macrophage antigen (Mac-2, 32000 dalton glycoprotein)
Biosafety:	1
Additional Info:	Spleen cells were fused with NS-1 myeloma cells. The Mac-2 antigen is not expressed on bone marrow cells. Like Mac-3, Mac-2 appears to be expressed on the monocytic line of differentiation at a stage after divergence from the granulocytic series. Mac-2 and Mac-3 are present on 69% of macrophages and 0% to 2% of thymocytes. Expression of Mac-2 is increased during the differentiation from monocyte to activated peritoneal macrophage.
Culture Medium:	RPMI-1640 medium modified to contain 2 mM L-glutamine, 1 mM sodium pyruvate, 4500 mg/L glucose and 10% of fetal bovine serum.
Subculturing:	Cultures can be maintained by the addition of fresh medium or replacement of medium. Adherent cells can be dislodged by scraping and cultures established by centrifugation with subsequent resuspension at 1 to 2 X 10 ⁵ viable cells/ml.



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Subculturing Medium Renewal:

Every 2 to 3 days

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

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:

Ho MK, Springer TA. MAC-2, a novel 32,000 Mr mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. J. Immunol. 128: 1221-1228, 1982. PubMed: 6173426 Springer TA. Monoclonal antibody analysis of complex biological systems. Combination of cell hybridization and immunoadsorbents in a novel cascade procedure and its application to the macrophage cell surface. J. Biol. Chem. 256: 3833-3839, 1981. PubMed: 7217058

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