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BCRJ Code: 0121

Cell Line: J774A.1

Species: Mus musculus

Vulgar Name: Mouse; Balb/C

Tissue: Blood

Cell Type: Monocyte; Macrophage

Morphology: Macrophage

Disease: Reticulum Cell Sarcoma

Growth Properties: Mostly Adherent

Sex: Female

Applications: Biological response transfection host

Products: Interleukin 1 (IL-1), lysozyme; IL5

Biosafety: 1

J774A.1 cells are active in antibody dependent phagocytosis [Pubmed: 1101071]. Their growth is inhibited by dextran sulfate, PPD and LPS [Pubmed: **Addtional Info:** 318922]. They synthesize large amounts of lysozyme and exhibits minor cytolysis but predominantly antibody-dependent phagocytosis. Interleukin 1

beta (II1b) is synthesized continuously by this line.

Dulbecco's Modified Eagle's Medium (DMEM) with 2 mM L-glutamine, 1.0 g/L **Culture Medium:**

glucose and 10% of fetal bovine serum.



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Subculturing:

remove old medium, add fresh, disloge cells by scraping, and dispense 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 Subcultivation Ratio:

io 1:3 to 1:6 is recommended.

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.

Thawing Frozen Cells:

- 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 $125 \times g$ 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).



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References:

Ralph P, et al. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. J. Exp. Med. 143: 1528-1533, 1976. PubMed: 1083890 Ralph P, Nakoinz I. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: 894031 Ralph P, Nakoinz I. Direct toxic effects of immunopotentiators on monocytic myelomonocytic, and histiocytic or macrophage tumor cells in culture. Cancer Res. 37: 546-550, 1977. PubMed: 318922 Sears DW, et al. Molecular cloning and expression of the mouse high affinity Fc receptor for IgG1. J. Immunol. 144: 371-378, 1990. PubMed: 2136886 Ralph P, et al. Reticulum cell sarcoma: an effector cell in antibodydependent cell- mediated immunity. J. Immunol. 114: 898-905, 1975. PubMed: 1089721 Ralph P, Nakoinz I. Phagocytosis and cytolysis by a macrophage tumour and its cloned cell line. Nature 257: 393-394, 1975. PubMed: 1101071 Knowlton KU, et al. A mutation in the puff region of VP2 attenuates the myocarditic phenotype of an infectious cDNA of the woodruff variant of coxsackievirus B3. J. Virol. 70: 7811-7818, 1996. PubMed: 8892902 Schissel SL, et al. Zn2+-stimulated sphingomyelinase is secreted by many cell types and is a product of the acid sphingomyelinase gene. J. Biol. Chem. 271: 18431-18436, 1996. PubMed: 8702487 Standard Practice for Testing for Biological Responses to Particles in Vitro. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 1903-98R03.

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