Code:	0154
Cell Line:	M3
Species:	Mus musculus **Vulgar Name:** Mouse, Balb/C
Tissue:	Mamarian Gland
Morphology:	Epithelial
Disease:	Adenocarcinoma
Growth Properties:	Adherent
Sex:	Female
Products:	GM-CSF; murine GM-CSG.
Biosafey:	1
Additional info:	This Cell line is derived from an adenocarcinoma of mammary glandthat arouse spontaneously in a BALB/c mouse. This carcinoma can be maintained in vivo by subcutaneously implant of 5x10E6 tumor cells: 100% mice will develop tumo and die after 45-50 days. More than 60% animals have metastases to lungs. This tumor has a low immunogenicity. The cell line M3 was adapted to in vitro culture. It secretes constitutively GM-CSF, and the supernatant of these cells sustain proliferation of GM-CSF dependent cell lines.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and fetal bovine serum to a final concentration of 10%.
Subculturing:	Remove medium, and rinse with PBS without calcium and magnesium. Remove the solution and add an additional 1 to 2 mL of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37°C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. T-75 flasks are recommended for subculturing this product. 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. **Medium Renewal:** 2 to 3 times per week **Subcultivation ratio:** 1:2 is recommende
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).
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