BCRJ Code:	0047
Cell Line:	BALB/3T3 clone A31
Species:	Mus musculus
Vulgar Name:	Mouse; Balb/C
Tissue:	Embryo
Cell Type:	Fibroblast
Morphology:	Fibroblast
Growth Properties:	Adherent
Age/Ethinicity:	EMBRYO: 14 to 17 days gestatio Day /
Applications:	This cell line is a suitable transfection host.
Virus Succeptility::	Herpes simplex virus Vesicular stomatitis virus
Tumor Formation::	No, in immunosuppressed mice Yes, in semisolid medium
Biosafety:	1
Addtional Info:	The cells are extremely sensitive to contact inhibition of cell division, grow at a high dilution, exhibit a low saturation density and are highly susceptible to transformation in tissue culture by the oncogenic DNA virus, SV40, and murine sarcoma virus.
Culture Medium:	Dulbecco's modified Eagle's medium with 4 mM L-glutamine with 4.5 g/L glucose and bovine calf serum to a final concentration of 10%. The serum used is calf serum, NOT fetal calf serum. The depositor recommended calf serum because fetal calf serum causes transformation and loss of contact inhibition.

Subculturing:	NOTE: Never allow cultures to become completely confluent before subculture. Some important considerations in the handling of 3T3 cells: doubling time is about 18 hours in sparse cultures. The cells reach a saturation density of about 10E6 cells per 20 cm2. In order to maintain this property of high contact inhibition, it is necessary to transfer routinely at only high dilutions, otherwise variants tend to be selected having reduced contact inhibition. Such low density makes culture vessels appear sparse and cell growth sensitive to sub-optimal temperature and media conditions. Volumes are given for a 75 cm2 flask. Increase or decrease the amount of dissociation medium needed proportionally 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
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO_2), 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:	22708: Aaronson SA, Todaro GJ. Development of 3T3-like lines from Balb-c mouse embryo cultures: transformation susceptibility to SV40. J. Cell. Physiol. 72: 141-148, 1968. PubMed: 4301006 26022: Todaro GJ, Aaronson SA. Properties of clonal lines of murine
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