BCRJ Code:	0017
Cell Line:	3T3-Swiss albino
Species:	Mus musculus
Vulgar Name:	Mouse
Tissue:	Embryo
Cell Type:	Fibroblast
Morphology:	Fibroblast
Growth Properties:	Adherent
Age/Ethinicity:	embryo /
Derivation:	The 3T3 cell line was established by G. Todaro and H. Green in 1962 from disaggregated Swiss mouse embryos.
Virus Succeptility::	POLYOMAVIRUS; SV40 virus
Products:	Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [PubMed: 9153219]
Biosafety:	1
Addtional Info:	The cells are contact inhibited. A confluent monolayer yields 40000 cells/cm2. The cells should be grown in plastic flasks, they do not grow well on some types of glass surfaces. A saturation density of approximately 50000 cells/cm2 can be reached.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified with 4500 mg/L glucose and bovine calf serum to a final concentration of 10%.

Subculturing:	NNever allow culture to become completely confluent. Subculture when 80% confluent or less. Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. 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. For 75 cm2 flasks use 4 X 105 cells per flask and subculture every 3 days. Population Doubling Time 18 hrs 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:	Twice a week
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:

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).

References:

Todaro GJ, Green H. Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. J. Cell Biol. 17: 299-313, 1963. PubMed: 13985244 Bennicelli JL, et al. Mechanism for transcriptional gain of function resulting from chromosomal translocation in alveolar rhabdomyosarcoma. Proc. Natl. Acad. Sci. USA 93: 5455-5459, 1996. PubMed: 8643596 Vogt M, Dulbecco R. Studies on cells rendered neoplastic by polyoma virus: the problem of the presence of virus-related materials. Virology 16: 41-51, 1962. PubMed: 13926482 Todaro GJ, et al. Antigenic and cultural properties of cells doubly transformed by polyoma virus and SV40. Virology 27: 179-185, 1965. PubMed: 4284655 Todaro GJ, et al. Transformation of properties of an established cell line by SV40 and polyoma virus. Proc. Natl. Acad. Sci. USA 51: 66-73, 1964. PubMed: 14104605 Fang X, et al. Lysophosphatidylcholine stimulates activator protein 1 and the c-Jun N-terminal kinase activity. J. Biol. Chem. 272: 13683-13689, 1997. PubMed: 9153219 Chen ST, et al. Generation of packaging cell lines for pseudotyped retroviral vectors of the G protein of vesicular stomatitiis virus by using a modified tetracycline inducible system. Proc. Natl. Acad. Sci. USA 93: 10057-10062, 1996. PubMed: 8816750 Campbell M, et al. The simian foamy virus type 1 transcriptional transactivator (Tas) binds and activates an enhancer element in the gag gene. J. Virol. 70: 6847-6855, 1996. PubMed: 8794326 Hsu DK, et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

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