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| Code: | 0017 |
| Cell Line: | 3T3-Swiss albino |
| Species: | Mus musculus Vulgar Name: Mouse |
| Tissue: | Embryo |
| Morphology: | Fibroblast |
| Growth Properties: | Adherent |
| Age Ethnicity: | embryo |
| Derivation: | The 3T3 cell line was established by G. Todaro and H. Green in 1962 from disaggregated Swiss mouse embryos. |
| Virus Susceptibility: | 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 |
| Additional info: | The cells are contact inhibited. A confluent monolayer yields 40000 cells/cm ² . 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/cm ² can be reached. |
| 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 bovine calf serum to a final concentration of 10%. |

Subculturing:

Never 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.

Medium Renewal: Twice per week

Subcultivation ratio:

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 highly recommended 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 submerged 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 it 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 an 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:

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 Benniselli 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 stomatitis 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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