Applied StemCell, Inc.
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Datasheet

Human Immortalized Fibroblasts

Product Information

Catalog Number	**ASE-9320**
Gender	Male
Age	45 years
Race	Caucasian
Clinical Information	Healthy
Tissue	Skin fibroblasts
Immortalization Method	Simian virus 40 (SV40) T antigen
Quantity	1 x 10^6 cells/vial
Shipping	Dry ice
Storage and Stability	Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.
Quality Control	By morphology; Fibroblast-like
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear the appropriate Personal Protective Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

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Media and Material

For 500 mL complete medium

- 430 mL ESC-Sure™ DMEM (Applied StemCell; Cat.# ASM-5001)
- 50 mL FBS (Applied StemCell; Cat.# ASM-5017)
- 5 mL 100x Non-Essential Amino Acids; 200 mM (ThermoFisher; Cat.# 11140-050)
- 5 mL 100x Sodium Pyruvate; 100 mM (ThermoFisher; Cat.# 11360-070)
- 5 mL 100x L-GlutaMax; 200 mM (ThermoFisher; Cat.# 35050-061)
- 5 mL 100x Penicillin-streptomycin; 5 mg/mL (ThermoFisher; Cat.# 15140-122)

Protocol

1. Thawing Procedure

1.1 Prepare a 75 cm^2 culture flask containing the complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
1.2 Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
1.3 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 operations from this point on should be carried out under strict aseptic conditions.
1.4 Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
1.5 Discard the supernatant and resuspend the cells in fresh growth medium. Add this suspension to the prepared culture vessel.
1.6 Incubate the culture at 37°C in a suitable incubator.
1.7 A 5% CO_2 /95% air atmosphere is recommended if using the medium described on this product sheet.

2. Culture Conditions

Note: Volumes recommended are for a 75 cm^2 flask. Increase or decrease the amount of disassociation medium needed proportionally for culture vessels of other sizes.

2.1 Remove and discard culture medium.
2.2 Add 3.0 to 5.0 mL of 0.25% trypsin-0.53 mM EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not hit or shake the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
2.3 Add 6.0 to 10.0 mL of complete growth medium and aspirate cells by gently pipetting.
2.4 Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 3 x 10^3 to 5 x 10^3 viable cells/cm^2 is recommended. Maintain cultures at a cell concentration between 8 x 10^3 and 1 x 10^4 cells/cm^2.
2.5 Passaging ratio: 1:2 to 1:3 twice weekly
2.6 Incubate cultures at 37°C.
2.7 Medium Renewal: every 2 to 3 days.

3. Cryopreserving Cells

Complete growth medium supplemented with an additional 60% fetal bovine serum and 10% DMSO.
Store in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.