

<b>Code:</b>	0114
<b>Cell Line:</b>	IB3-1 [JHU-52]
<b>Species:</b>	Homo sapiens  <b>Vulgar Name:</b> Human
<b>Tissue:</b>	lung
<b>Morphology:</b>	Epithelial
<b>Disease:</b>	Fibrosis
<b>Growth Properties:</b>	Adherent
<b>Sex:</b>	Male
<b>Derivation:</b>	IB3-1 is an immortalized cell line created in 1992 from a primary culture of bronchial epithelia cells isolated from a patient with cystic fibrosis. The culture was transformed with a hybrid virus, adeno-12-SV40 [PubMed: 1849726]
<b>Applications:</b>	Transient Transfection
<b>Virus Susceptibility:</b>	CELLS CONTAIN SV40 AND ADENOVIRUS 12 DNA VIRAL SEQUENCES
<b>Biosafety:</b>	2
<b>Culture Medium:</b>	LHC-8 Basal Medium (Invitrogen catalog #12679-015 ), 95%; fetal bovine serum, 5%

**Subculturing:**

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. Is recommended Maintain cultures at a cell concentration between  $4 \times 10^3$  and  $4 \times 10^4$  cells/cm<sup>2</sup> 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:**

**Culture Conditions:**

Atmosphere: air, 95%; carbon dioxide (CO<sub>2</sub>), 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 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 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:**

39291: Flotte TR, et al. Gene expression from adeno-associated virus vectors in airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 7: 349-356, 1992. PubMed: 1325813  
70684: Afione SR, et al. Expression of the cystic fibrosis transmembrane conductance

**Depositors:**

Luiz Soares; Grupo Boticario.

**ATCC:**

CRL-2777