

## B-ALI™ Immune-Airway Co-Culture Model: Normal Human Bronchial Epithelial Cells and Peripheral Blood Mononuclear Cells

### Instructions for use

---

#### Safety Statements

These products are not for use in GMP manufacturing, nor human or animal in vivo use, including use as a diluent or as an excipient, or for diagnostic use.

These products are for research use *only*.

**WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS.** Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories, 5th edition](#). If you require further information, please contact your site safety officer or Scientific Support.

#### Preparation of reagents

All work should be performed in a laminar flow hood. Decontaminate the external surfaces of all supplement vials and the medium bottles with ≥70% ethanol or isopropanol.

1. **B-ALI™ Growth Medium and B-ALI™ Differentiation Medium**  
Prepare Growth Medium and Differentiation Medium according to instructions in the [B-ALI™ Bronchial Air-Liquid Interface Medium Instructions for Use](#) guide found on the Lonza

website. NOTE: the Retinoic Acid and Inducer supplements are light sensitive. Do not add inducer to the Differentiation Medium until you are ready to use the medium (must be thawed from frozen single aliquots and prepared fresh for each media change).

2. **50/50 Co-Culture Medium**
  - a. Determine the total volume of 50/50 Co-culture Medium needed (e.g., 500 µL per well in a 24-well Corning® Transwell® plate; Corning® P/N 3470). You will need to change medium at least once during coculture.
    - i. This protocol was tested to 4 days of co-culture. We recommend changing media every 2 days.
  - b. Prepare all media on day of co-culture establishment and refrigerate surplus media for up to 2 weeks at 4°C in aliquots for later use.
  - c. Create the required amount of **50/50 basal medium** by combining X-VIVO® 15 Serum-free Hematopoietic Cell Medium and B-ALI™ Differentiation Basal Medium (medium **without SingleQuotes® added**) in a 1:1 ratio.
  - d. Add B-ALI™ SingleQuotes® supplements **except for the Inducer** to 500 mL of the 50/50 basal medium following the instructions in the table below:

SingleQuots® Supplements	Volume to add to 500 mL 50/50 basal medium
BPE	2.0 mL
Insulin	0.5 mL
Hydrocortisone	0.5 mL
GA-1000	0.5 mL
Retinoic Acid	0.5 mL
Transferrin	0.5 mL
Triiodothyronine	0.5 mL
Epinephrine	0.5 mL
hEGF	0.5 mL

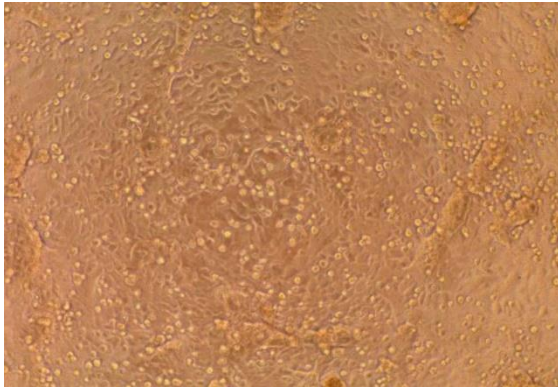
- i. Adjust volumes of supplements according to the total volume of 50/50 basal medium used.
- e. Resuspend 10 µg IL-2 IS in 1000 µL PBS for a final concentration of 500X IL-2.
  - i. Final concentration in 50/50 Co-Culture Medium should be 50 – 100 IU/mL; adjust stock concentration as needed.
- f. Add an appropriate volume of 500X IL-2 to the 50/50 medium to make a final concentration of 1X IL-2.
- g. Aliquot medium to be used for future media changes and store at 4°C for up to 2 weeks.
- h. To the remaining medium, add 20 µL B-ALI™ Inducer supplement (found in the B-ALI™ SingleQuots® Kit) per 10 mL 50/50 Co-Culture Medium.
- i. For each media change, use a single aliquot of 50/50 Co-Culture Medium and add the inducer as above immediately prior to use.

## Normal Human Bronchial Epithelial (NHBE) Cell Culture and Airlift

**NOTE:** All work is to be performed in a laminar flow hood.

1. Add 25 mL of B-ALI™ Growth Medium to a T-75 flask and allow the vessel to equilibrate in a 37°C, 5% CO<sub>2</sub> humidified incubator for at least 30 minutes.
2. Wipe cryovial of NHBE cells with ethanol or isopropanol before opening. In a sterile hood, briefly twist cap to relieve pressure and then re-tighten. Thaw the cryovial in a 37°C water bath. **DO NOT SUBMERGE.** Remove when a sliver of ice remains (takes less than 2 minutes).
3. Resuspend the cells in the cryovial.
4. Pipette all cell suspension into the T-75 flask from step 1. Gently rock to distribute cells and return to incubator.
  - a. **DO NOT CENTRIFUGE CRYOVIAL.** This damages the cells more than DMSO residue in culture.
5. Change media on Day 1, using 25 mL pre-warmed B-ALI™ Growth Medium.
6. Prepare collagen-coated plates on the day of NHBE harvest (when confluence is between 50 – 90%; 2 – 4 days after seeding).
  - a. Dilute stock collagen with PBS to make a 30 µg/mL collagen solution. Make enough solution to add 100 µL per Transwell®.
  - b. Coat plate inserts with 100 µL of collagen solution per well. Place plate in the incubator for 45 minutes.
  - c. Aspirate the collagen solution from each well.
  - d. Gently wash each well with 150 µL PBS and aspirate off liquid.
  - e. Leave the dry plate in the hood until cells are added. Do not let the plate sit overnight.
7. Harvest NHBE cells according to the instructions below:
  - a. Warm 5mL trypsin/EDTA to 37°C in a water bath.
  - b. Allow 10 mL HEPES buffered saline (HEPES-BSS) to come to room temperature.
  - c. Allow 5 mL of trypsin neutralizing solution (TNS) to come to room temperature.
  - d. Warm up 15 mL of B-ALI™ Growth medium to 37°C in the water bath.
    - i. Do not warm up the entire bottle, just warm 15 mL for each 24-well plate.
  - e. Aspirate medium from the T-75 flask with NHBE cells.
  - f. Rinse cells with 10 mL room-temperature HEPES BSS.
  - g. Aspirate the HEPES-BSS from the flask.
  - h. Cover cells with 5 mL trypsin/EDTA.
  - i. When 90% of cells have rounded up (takes 2 – 6 minutes), gently tap the flask against the palm of your hand to release the majority of cells.
    - i. If cells do not detach, wait another 30 seconds and tap again. Repeat until cells detach.
  - j. Add 5 mL trypsin neutralizing solution.
  - k. Quickly transfer detached cells to a 15 mL centrifuge tube.
    - i. If, after 6 minutes, the majority of cells haven't detached, harvest detached cells and then repeat steps h-k.
  - l. Rinse flask with a final 2 mL of HEPES-BSS to collect residual cells and add rinse to the 15 mL centrifuge tube.

- m. Centrifuge harvested cells at 200xg for 5 minutes.
- n. Aspirate supernatant except for 100 – 200  $\mu$ L.
8. Resuspend pellet in 1 mL B-ALI™ Growth Medium and count a 20  $\mu$ L aliquot with Trypan Blue 0.4% and a hemocytometer. We recommend using a 1:2 dilution of Trypan Blue (e.g., 20  $\mu$ L sample + 20  $\mu$ L Trypan Blue).
9. Dilute cells to an appropriate concentration using B-ALI™ Growth Medium (e.g., 500,000 cells/mL) and then seed on the collagen-coated Corning® 24-well Transwell® membrane plate at a density of 50,000 cells/well.
10. Add 500  $\mu$ L B-ALI™ Growth Medium to the basal chamber and culture in the incubator for 3 – 4 days.
  - a. Change media after 1 day and again on day 3 if cells are not showing the morphology displayed in Figure 1.
  - b. Check morphology and confluence daily. Proceed to airlift step on day 3 or day 4, whenever the cell layer displays the proper morphology (Figure 1).
  - c. Airlift step: On day 7 – 8 (3 – 4 of Transwell® culture), remove media from both sides of the membrane. Add 500  $\mu$ L **B-ALI™ Differentiation Medium** with **freshly thawed inducer** to the basal media chamber underneath the membrane.
- a. Lonza offers ampules of  $5 \times 10^7$  (CC-2702) and  $1 \times 10^8$  (CC-2703) PBMCs.
- b. Thaw more or fewer cells depending on the total # needed – each plate well will receive  $5 \times 10^5$  PBMCs.
2. Pipette 25.5 mL of pre-warmed X-VIVO® 15 Serum-free Hematopoietic Cell Medium into a 50 mL conical tube.
3. Pipette cells into 50 mL conical tube of X-VIVO® 15 from step 2.
4. Wash cell vial(s) with 1 mL X-VIVO® 15 and add wash to 50 mL conical tube.
5. Centrifuge cells at 300xg for 10 minutes at room temperature.
6. Aspirate supernatant without disturbing cells and resuspend pellet in 2-5 mL **50/50 Co-Culture Medium (remember to add inducer – see reagent preparation steps)**.
7. Count an aliquot of cells using Trypan Blue (0.4%) and a hemocytometer (1:2 dilution; e.g., 20  $\mu$ L sample + 20  $\mu$ L Trypan Blue).
8. Dilute cells to a final concentration of  $1 \times 10^6$  cells/mL.
9. Remove media from the basal chambers of the Transwell® plate.
10. Add 500  $\mu$ L of PBMC cell suspension ( $5 \times 10^5$  PBMCs) to the basal chamber of each well with desired treatment conditions added to the media.
  - a. For a positive T cell activation control, add 25  $\mu$ L of ImmunoCult™ anti-CD3/CD28 antibody (STEMCELL™ Technologies) per mL of media to the 50/50 Co-Culture media before resuspending cells and adding 500  $\mu$ L per positive control well.
11. Incubate co-culture at 37°C, 5% CO<sub>2</sub> for 4 days (counting day of co-culture establishment).
12. Change medium 2 days after plating:
  - a. Remove medium and suspended PBMCs from each well and place in a separate, labeled microcentrifuge tube.
  - b. Refill basal chamber of each well with 500  $\mu$ L PBS.
  - c. Spin each tube at 300xg for 5 minutes.
  - d. Remove supernatant from each microcentrifuge tube without disturbing cells.
  - e. Resuspend cells in 500  $\mu$ L prewarmed 50/50 Co-Culture Medium **with inducer, treatment conditions, and ImmunoCult™ anti-CD3/CD28 antibody stimulator added where appropriate**.
  - f. Remove PBS from the basal chamber of each well and replace PBMCs back in appropriate wells.



**Figure 1: NHBE morphology before airlift, day 3-4.**

11. **Culture for 14 more days** at 37°C, 5% CO<sub>2</sub>, performing media changes (basal media chamber only) every 2 – 3 days.

12.

#### **NHBE: PBMC Co-Culture Establishment**

1. On day 14 after airlifting the NHBE cells, thaw  $1 \times 10^8$  Lonza Peripheral Blood Mononuclear Cells (PBMCs) at 37°C until a sliver of ice remains.

13. A variety of analyses can be performed on this co-culture to examine impacts of experimental treatments:
- NHBE membrane health can be assessed by:
    - TransEpithelial Electrical Resistance (TEER) – a measure of membrane integrity.
    - Mucin staining (mucin production; a measure of NHBE cellular differentiation).
    - $\beta$ -tubulin staining (a measure of membrane integrity and cilia formation).
    - Membrane sectioning and hematoxylin + eosin (H&E) staining (to measure membrane thickness and assess overall integrity visually).
  - PBMC population viability and functionality can be assessed via immunophenotyping (flow cytometry). For example, a panel of antibodies including CD3, CD4, CD8, CD25, CD45-RA and CD45-RO can be used to analyze the T cell population for T cell activation and assess sub-population sizes of differentiated T cells (helper, cytotoxic, etc.)
14. Applications for this model include effects of inflammation, respiratory diseases, drugs, and environmental contaminants on airway health and functioning.
- NOTE: In this model, PBMCs do not come into full contact with NHBE cells because of the Transwell® membrane. Therefore, this model is more suited to study necrotic and inflammatory effects of coculture than effects induced by T-cell receptor binding.

00193514	B-ALI™ Bronchial Air-Liquid Interface Medium BulletKit®	Contains Growth Basal Medium, Differentiation Basal Medium, and SingleQuots® supplement kit
04-418Q, 02-060Q (EU and ROW)	X-VIVO® 15 Serum-free Hematopoietic Cell Medium	1 L, complete with L-Glutamine, gentamicin, and phenol red; xenofree
CC-5024	HEPES Buffered Saline Solution (HEPES-BSS)	500 mL bottle
CC-5012	Trypsin/EDTA	100 mL bottle
CC-5002	Trypsin Neutralizing Solution (TNS)	100 mL bottle

## THESE PRODUCTS ARE FOR RESEARCH USE ONLY.

Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures. Not for use in diagnostic procedures.

PBS without Calcium or Magnesium (ThermoFisher Scientific 10010023) mentioned is a product of GIBCO™.

IL-2 IS (Miltenyi 130-097-743) mentioned is a product of Miltenyi Biotec.

Collagen (rat tail collagen type I; Corning® 354236) mentioned is a product of Corning®.

Trypan Blue 0.4% (ThermoFisher 15250061) mentioned is a product of GIBCO™.

24-well Transwell® plates (Corning® 3470) mentioned are a product of Corning®.

ImmunoCult™ CD3/CD28 antibody (STEMCELL™ Technologies 10991) is a product from STEMCELL™ Technologies.

All trademarks belong to Lonza, and are registered in USA, EU or CH or belong to third party owners and are used only for informational purposes. All third party copyrights have been reproduced with permission or belong to Lonza. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group Ltd or its affiliates and the information and recommendations given by Lonza Group Ltd or its affiliates are (i) suitable for intended process or purpose, (ii) in compliance with environmental, health and safety regulations, and (iii) will not infringe any third party's intellectual property rights. The user bears the sole responsibility for determining the existence of any such third party rights, as well as obtaining any necessary licenses. For more details: [www.lonza.com/legal](http://www.lonza.com/legal). ©2023 Lonza. CD-OP016 07/23

## Ordering Information

Catalog No.	Description	Size
CC-2540S	Cryopreserved Human Normal Bronchial Epithelial (NHBE) Cells for B-ALI™ culture	≥ 500,000 cells
CC-2702, CC-2703, CC-2705	Human Peripheral Blood Mononuclear Cells (HPBMC)	≥ 50 million cells, ≥ 100 million cells, ≥ 25 million cells
00193516	B-ALI™ Growth Basal Medium	250 mL
00193517	B-ALI™ Differentiation Basal Medium	500 mL
00193515	B-ALI™ SingleQuots® Supplement Kit	1 kit