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www.bioscience.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
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Lonza Normal Human Intestinal Epithelial Cell Systems

Instructions for use

Safety statements

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.

WARNING: LONZA PRODUCTS 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-I, 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, hepatitis B virus, and hepatitis C virus. Testing can not 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 of 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.

Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact customer service.
3. For proliferating cells – swab down the flask of proliferating cells with 70% ethanol or isopropanol, then place the flask in 37°C, 5% CO_2, humidified incubator and allow to equilibrate for three to four hours. After cells have equilibrated, remove shipping medium from the flask and replace with fresh medium.
4. BulletKit® Instructions: upon arrival, store basal medium at 4-8°C and SingleQuots® at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4°C and added to the basal medium within 72 hours of receipt. After SingleQuots® are added to basal medium, use within one month. Do not re-freeze.

Preparation of media

For a BulletKit®, perform the following steps:

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire amount to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label (avoid covering the basal medium lot # and expiration date) to avoid confusion or possible double supplementation.
5. Record the new expiration date on the label based on the shelf life.

NOTE: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be refiltered with a 0.2 µm filter to assure sterility. Routine refiltration is not recommended.

Collagen coating of tissue culture vessels

1. For primary human intestinal epithelial cells (InEpC) to effectively attach and spread on tissue culture dishes, rat-tail type 1 collagen coating of the surfaces is **strongly recommended**.
2. To prepare coating solution, dilute commercially available stock of rat-tail type 1 collagen (BD Biosciences No. 35-4236) in PBS-1X, w/o Ca++, Mg++ to a concentration of 30 µg/mL and add

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enough solution to completely cover the surface of each well (200-250 µL per well for 48 well plate or 50-75 µL of the solution per 24-well transwell insert).

3. Incubate plates with their covers on for at least 60 minutes at room temperature or 30 minutes at 37°C.
4. Aspirate the collagen solution, wash each well once with 2-3 volumes of PBS, and let plates dry for 5-10 minutes at room temperature.
5. Use immediately or seal plate in a resealable bags and store upside down at 4°C for **no more then 1-2 days**.

Thawing of cells

1. The recommended seeding density of InEpC in tissue culture dishes is 150,000 viable cells/cm^2.
2. To set up cultures, calculate the number of wells needed and cover the surface area with rat-tail type 1 collagen as described earlier.
3. Prepare one "centrifugation tube" for each cryovial immediately before or while thawing the cells. To do so, add 5 mL of SmGM®-2 Medium into a 15 mL centrifuge tube. Slowly add 1 mL of regular FBS along the wall of the tube on top of the media to create a gradient. **Do not use heat inactivated FBS.**
4. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten.
5. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
6. Slowly, along the wall of the tube, add the cell suspension to the centrifugation tube. Wash the cryovial with 1 mL of SmGM®-2 Media and add the wash to the centrifugation tube.
7. Centrifuge the tube(s) at **440-500 g** for 6 minutes at room temperature. Usually, clearly visible sheets of cells are concentrated close to the bottom of the tube on top of FBS.
8. **Resuspend cells back** into the medium by inverting tube 2-3 times and repeat centrifugation as describe in step 7. Cell should form a compact pellet after this step.
9. Carefully aspirate all but 150-200 µL of supernatant on top of the cell pellet. Resuspend cells into remaining medium by flicking the bottom of the tube 5-6 times.
10. Add 0.5 mL of SmGM®-2 Media using 1 mL pipette and gently resuspend the cells until they form homogenous suspension with no or very few cell aggregates. If thawing several cryovials at the same time, combine cells into one pool at this stage.
11. Add 1 mL of SmGM®-2 Media to the cells to generate 1.5 mL of cell suspension for each cryovial.
12. Reference the certificate of analysis supplied with the cells to calculate a concentration of viable InEpC in the suspension.

Plating InEpC into tissue culture dishes

NOTE: The following instructions are for a 48 well plate (~1 cm^2 of plating surface per well). Adjust all volumes accordingly for other size dishes.

1. Precoat the necessary amount of wells with rat-tail type 1 collagen as previously described.
2. Add 150 µL of SmGM®-2 Media per well.
3. Mix InEpC suspension by inverting and flicking the tube several times and add the calculated volume of cell suspension containing ~150,000 viable InEpC per well.

NOTE: If optimizing the plating cell density, vary the amount of viable cells per well within a range of 150,000±50,000 viable cells per well keeping the total volume of cells and media within a range of 300±50 µL per well.

4. Incubate cells **in CO_2 incubator at 33°C** overnight.
5. Change the media next day and every other day after that. Continue incubation at **33°C**. Cells will be at ≥50% confluency on day 5-7 after plating.
6. One vial of cells should yield ≥ 6 wells of 48 well plate with confluency ≥ 50%. For more precise results, optimization of plating cell density is recommended for each lot of InEpC.

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Plating InEpC into filter plates (co-culture with InMyoFib)

InEpC form tight monolayer with high transepithelial electrical resistance (TEER, up to 10,000 Ω per well) when co-cultured with human intestinal myofibroblasts (InMyoFib).

NOTE: The following instructions are for a 0.33 cm^2 transwell inserts (Costar PET transwell plates, Corning No. 3470). Adjust all volumes accordingly for other size transwell plates.

1. Establish culture of InMyoFib as recommended in Lonza's normal human intestinal myofibroblast cell system - Instructions for use."
2. When subculturing myofibroblasts, plate ~100,000 myofibroblasts per well in the basal compartment of a 24-well transwell plate in 1 mL of SmGM®-2 Media for 1-3 days prior plating InEpC.
3. Precoat necessary amount of transwell inserts with rat-tail type 1 collagen as previously described.
4. Thaw and resuspend InEpC in 1.5 mL of SmGM®-2 Media per cryovial as previously described.
5. Insert transwell inserts into 24-well transwell plates containing InMyoFib in the basal compartment.
6. Mix InEpC suspension by inverting and flicking the tube several times and add calculated volume of cell suspension containing ~100,000 viable InEpC per insert in the apical chamber of each well (the InMyoFib in the corresponding basal compartment).
7. Incubate cells **in CO_2 incubator at 33°C** overnight.
8. Change the media in the apical chamber the next day and in both chambers every other day after that. Continue incubation at **33°C**. Typically, cells will reach maximum TEER on days 6-7 after plating.
9. One vial of cells should yield ≥ 8 inserts of a 24-well transwell plate with TEER values ≥ 2,000 Ω per well. Plating ~150,000 viable InEpC per filter usually accelerates maximum TEER formation by 2-3 days. Optimal amount of InEpC per filter, time and value of TEER should be determined experimentally for each lot of frozen InEpC.

Ordering information

Cryopreserved cells (single donor)		
CC-2931	Human intestinal epithelial cells	≥ 800,000 viable cells
CC-4540	Human intestinal cell co-culture combo	Contains one ampule of InEpC (≥ 800,000 viable cells) and one ampoule of InMyoFib (≥ 500,000 cells)

Freshly plated cells are also available for purchase by calling customer service.

Related products

Culture media (must be purchased separately):		
CC-3182	SmGM®-2 BulletKit®	Kit which contains a 500 mL bottle of SmBM (CC-3181), and SmGM®-2 SingleQuots® (CC-4149).
CC-3181	SmBM®-2 Media	SmBM smooth muscle cell basal medium (no growth factors) (500 mL)
CC-4149	SmGM®-2 SingleQuots® formulates SmBM®-2 Media to SmGM®-2 Media	Supplements and growth factors (insulin, hFGF-B, hEGF, FBS and gentamicin/ amphotericin-B)
FBS	US Origin	500 mL
CC-2902	InMyoFib	≥ 500,000 cells

Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza warrants its cells only if Lonza media and reagents are used.

Quality control

HIV-1, hepatitis B and hepatitis C are not detected for all donors and/or cell lots. Routine characterization of InEpC includes viability, plating efficiency and immunofluorescent staining. For detailed information concerning QC testing, please refer to the certificate of analysis.

When placing an order or for technical support, please refer to the product numbers and descriptions listed above. For a complete listing of

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