Human Lung Epithelial Stem Cells

Catalog No.	Product Name	Product quantity	Storage on Arrival	Thawing Instructions	Storage once Thawed
ax3005	Lung Epithelial Stem Cells	500,000 cells/vial	Liquid nitrogen	Follow protocol	N/A
ax3580	Lung Epithelial Stem Cell Culture Medium	500 mL	Aliquot and store at -20°C for up to 6 months	Thaw at 4°C or RT	Store at 4°C for up to 1 month
ax0047	SureGrowth Recombinant Human FGF2	100 µg lyophilized powder	Store at -20°C	Reconstituted protein should be used immediately or stored in working aliquots at -20°C	Store at -20°C
ax0047X	SureGrowthX Recombinant Human EGF	100 µg lyophilized powder	Store at -20°C		Store at -20°C
ax0044	Unlock	25 mL	Aliquot and store at -80°C for up to 6 months	Thaw at 4°C or RT	Store at 4°C for up to 1 week
ax0041	SureBond	3 x 120 µL	Store at -80°C	Thaw at 4°C	Store at 4°C for up to 2 weeks
ax0049	Fibronectin Coating Solution	1 mL	Aliquot and store at -80°C	Thaw at 4°C	Once diltued, use immediately

Lot-specific information is stated in the Certificate of Analysis.

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Fetal Bovine Serum	Multiple	N/A

Recommendations:

Always count the number of viable cells after thawing

- Recommended cell culture medium: **Lung Epithelial Stem Cell Culture Medium + 10 ng/mL EGF + 10 ng/mL FGF2**
- Recommended incubation conditions: **37°C, 7% CO_2**
- Recommended centrifugation speed: **200 x *g* for 5 minutes**

Address: Axol Bioscience Limited, Suite 3, The Science Village, Chesterford Research Park, Little Chesterford, Cambridgeshire, CB10 1XL
Phone: +44 (0) 1223 751 051 | US Toll Free Tel: 1-800-678-2965
Email: support@axolbio.com
Web: www.axolbio.com

Preparation of Growth Factors:

- Prepare **100 µg/mL** stock solutions for **SureGrowth Recombinant Human FGF2** and **SureGrowthX Recombinant Human EGF** by resuspending 100 µg of lyophilized powder in 1 mL of sterile PBS supplemented with 0.1% human serum albumin.
- Aliquot into working volumes and store at **-20°C**.

Thawing, Plating & Expansion:

- Transfer the vial of cells from liquid nitrogen storage with the vial buried in dry ice. Remove the vial from dry ice and transfer it immediately to a **37°C** water bath.
- Thaw the cells quickly in a **37°C** water bath until just prior to complete thawing.
- Wipe the outside of the vial with 70% ethanol.
- Gently resuspend the cells and transfer to a 15 mL sterile conical tube.
- Slowly add **10 mL** of pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium**.
- Rinse the cryovial with 1 mL of medium to ensure all of the cells are transferred.
- Centrifuge the cells at **200 x *g*** for **5 minutes**.
- Carefully remove the supernatant and resuspend the cell pellet in 1 mL of pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 10 ng/mL EGF + 10 ng/mL FGF2**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 10 ng/mL EGF + 10 ng/mL FGF2**.
- Seed cells into the culture vessel at the required seeding density.
- Incubate the cells at **37°C**, **7% CO_2** in a humidified incubator.
- The cells grow in suspension as aggregates in the serum-free cell culture medium.
- Replace half the volume of culture medium **every 2 days** with fresh pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 10 ng/mL EGF + 10 ng/mL FGF2**.

Allow the cells to settle at the bottom of the culture vessel before replacing half of the medium volume. If the cell aggregates do not settle or cannot be seen by eye, centrifuge the cell suspension and replace the culture medium.

Address: Axol Bioscience Limited, Suite 3, The Science Village, Chesterford Research Park, Little Chesterford, Cambridgeshire, CB10 1XL
Phone: +44 (0) 1223 751 051 | US Toll Free Tel: 1-800-678-2965
Email: support@axolbio.com
Web: www.axolbio.com

Passaging:

- Passage when the suspension culture is near to confluency or when the cells exhaust the culture medium rapidly as determined by phenol red color change to yellow indicating acidification of the culture medium.
- Recommended passaging reagent: **Unlock**

- Collect the cell suspension in a sterile conical tube.
- Centrifuge the cells at **200 x *g*** for **5 minutes**.
- Gently discard the supernatant and resuspend the cell pellet in **10 mL** of D-PBS.
- Centrifuge the cells at **200 x *g*** for **5 minutes**.
- Gently discard the supernatant and resuspend the cell pellet in **~5-10 mL** of **cold/room temperature** **Unlock** passaging reagent.
- After adding passaging reagent, incubate the cells for **5 minutes** at **37°C**.
- Dilute out the passaging reagent with four volumes of pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium**. (For example if 1 mL of **Unlock** is used, then add 4 mL of the medium to stop the reaction). Gently pipette up and down a few times to disperse the medium.
- Centrifuge the cells at **200 x *g*** for **5 minutes**.
- Carefully remove the supernatant and resuspend the cell pellet in **2 mL** of pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 10 ng/mL EGF + 10 ng/mL FGF2**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 10 ng/mL EGF + 10 ng/mL FGF2**.
- Seed cells into the culture vessel at the required seeding density.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator.
- Replace half the volume of culture medium **every 2 days** with fresh pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 10 ng/mL EGF + 10 ng/mL FGF2**.

Address: Axol Bioscience Limited, Suite 3, The Science Village, Chesterford Research Park, Little Chesterford, Cambridgeshire, CB10 1XL
Phone: +44 (0) 1223 751 051 | US Toll Free Tel: 1-800-678-2965
Email: support@axolbio.com
Web: www.axolbio.com

Differentiation *in vitro*:

- Coat the culture vessel with **SureBond** (ax0041; Laminin Coating Solution) or **Fibronectin Coating Solution** (ax0049).
- **SureBond** biases differentiation to alveolar cell types whereas **Fibronectin** biases differentiation to bronchiolar cell types.

SureBond Instructions:

- Dilute the **SureBond** stock solution **1:50** in sterile Dulbecco's-PBS (D-PBS, without calcium or magnesium) to make 1x working solution e.g. **120 µL** in **6 mL**.
- Coat the surface of your culture vessel with the **SureBond** 1x working solution. We recommend coating at a volume of **200 µL per cm^2** however, please optimize for your experiments.
- Incubate the culture vessel **overnight** at **37°C** in a humidified incubator.

Fibronectin Coating Solution Instructions:

- Dilute the stock **Fibronectin Coating Solution 1:100** in sterile water to make 1x working solution e.g. **100 µL** in **10 mL**.
- Coat the surface of your culture vessel with the **Fibronectin** 1x working solution. We recommend coating at a volume of **200 µL per cm^2** however, please optimize for your experiments.
- Incubate the culture vessel **overnight** at **37°C** in a humidified incubator.

Differentiation:

- The extracellular matrix (ECM) coating and the addition of fetal bovine serum (FBS) to the culture medium will induce differentiation of the stem cells.
- Seed the cells into the **coated** culture vessel in pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 2% FBS + 10 ng/mL EGF + 10 ng/mL FGF2**.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator.
- Replace the culture medium **every 3 days** with fresh pre-warmed, **37°C**, **Lung Epithelial Stem Cell Culture Medium + 2% FBS + 10 ng/mL EGF + 10 ng/mL FGF2**.
- Differentiation will occur over **10-15 days**.

Address: Axol Bioscience Limited, Suite 3, The Science Village, Chesterford Research Park, Little Chesterford, Cambridgeshire, CB10 1XL
Phone: +44 (0) 1223 751 051 | US Toll Free Tel: 1-800-678-2965
Email: support@axolbio.com
Web: www.axolbio.com

Usage Statement:

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
International phone **+44-1223-751-051**
US phone **+1-800-678-AXOL (2965)**

Address: Axol Bioscience Limited, Suite 3, The Science Village, Chesterford Research Park, Little Chesterford, Cambridgeshire, CB10 1XL
Phone: +44 (0) 1223 751 051 | US Toll Free Tel: 1-800-678-2965
Email: support@axolbio.com
Web: www.axolbio.com