3DProSeed™ StromaLine Lung Cancer-Associated Fibroblasts

(Catalog Number: ECT.STRL.LUNCAF.096)

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

Contents and storage

Product	Part number	Quantity	Storage	Description
StromaLine Lung CAF	ECT.STRL.LUNCAF.096/01	2x vial, 10^6 cells	Liquid N_2	Cryopreserved human CAF isolated from invasive squamous cell carcinomas of the lung
StromaLine Lung CAF Medium	ECT.STRL.LUNCAF.096/02	2x kits basal medium with supplements)	2-4 °C	Culture medium optimized for the growth of the StromaLine Lung CAF
StromaLine LungCAF 3DProSeed hydrogel Assay Plate	ECT.STRL.LUNCAF.096/03	1x96-well hydrogel plate	RT in correct orientation	96-well glass-bottom hydrogel plate optimized for the growth of the StromaLine Lung CAF

Product Overview

The 3DProSeed™ StromaLine Lung Cancer-Associated Fibroblasts (CAF) is a pre-developed human stromal model made of patient-derived Lung stellate CAF in synthetic, optically transparent 3D hydrogels. The platform is optimized for generating relevant 3D stromal cultures enabling the sequential addition of tumor cells. It allows the study of the assembly and deposition of a native extracellular matrix resembling the stromal component of the tumor microenvironment of human Lung cancers. The hydrogel is optically transparent and pre-casted in a 96-well imaging plate, allowing a wide range of microscopy and high-content assays. Additionally, the hydrogel can be enzymatically dissolved at the end-point of the culture, and the cells, as well as the extracellular matrix fraction, can be retrieved and processed for further biochemical analysis, including proteomics and transcriptomics analyses. The cells can be delivered directly into your laboratory pre-plated (growing in the hydrogel plate) or cryopreserved (ready for seeding in the hydrogel plate whenever needed). The 3DProSeed StromaLine cells, medium, and hydrogel plate are quality tested together and guaranteed to give optimum performance as a complete system.

Seeding Protocol for Cryopreserved Cells

1. Bring the StromaLine Lung CAF Assay Plate at room temperature and the StromaLine Lung CAF Medium at 37 °C for at least 30 min prior to use.
2. Thaw the frozen StromaLine Lung CAF vial directly upon arrival or after storing in liquid N_2 in a 37 °C water bath for a maximum of 90 sec.
3. Carefully mix the cell suspension at least 20x with a P1000 pipet and transfer to a 50-mL conical tube containing 20 mL of StromaLine Lung CAF Medium, pre-warmed at 37 °C. **Do not centrifuge the cell suspension.** Centrifugation leads to decreased cell numbers and viability. This step generates a cell suspension with a density of 100,000 cells/mL, sufficient to seed the entire 96-well plate. We recommend this density for optimal results.
4. Carefully peel off the sealing adhesive foil from the StromaLine Lung CAF Assay Plate. A liquid meniscus may form on top of the wells due to the negative pressure applied by removing the foil, but it pops and disappears within seconds. Using a P100 pipet, insert the pipet tip in the well and descend along the side wall until you reach the plastic ring inside the well. Aspirate carefully the storage saline buffer. Do not touch or aspirate

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right over the hydrogel to prevent damaging it. Avoid aspirating the storage buffer using a vacuum pump as the suction force may damage the hydrogel. The storage buffer is a Tris-based buffer, and if some left-over remains in the well, it will not negatively affect culture development.

5. Add 200 µL/well of the cell suspension prepared in step 3 to the StromaLine Lung CAF Assay Plate. This will achieve a cell density of 20,000 cells/well, which we recommend for optimal results. Maintain the culture in a 37 °C humidified incubator under a 5% CO_2 atmosphere. Change the medium every 2-3 days (200 µL/well). We recommend aspirating the medium using a multichannel pipet and not a vacuum pump as the suction force may damage the hydrogel. The culture can be maintained for at least 12 days.

More detailed protocols for handling the assay plate and for cell seeding can be found in the 3DProSeed General Usage Manual (available upon request).

Cell Characterization

The 3DProSeed StromaLine Lung CAF system has been characterized by bright-field microscopy to ensure cell penetration into the hydrogel and growth of spindle-like, fibroblastic cells into 3D networks (Figure 1). The expression of the CAF marker alpha-smooth muscle actin (α-SMA) by cells grown in the 3DProSeed StromaLine Lung CAF system is assessed by immunofluorescence (Figure 2).

Figure 1. Representative field of view of the 3DProSeed StromaLine Lung CAF culture. Bright-field images were acquired at different focal planes (218, 435, and 544 µm from the glass bottom respectively), showing a 3D cellular network spanning a volume of ~300 µm. Such cell morphology and density can be achieved within 10 days of culture following the protocol delineated in section 3. The images were acquired with a Cytation1 BioTek imager at 4x magnification. Scale bar: 200 µm.

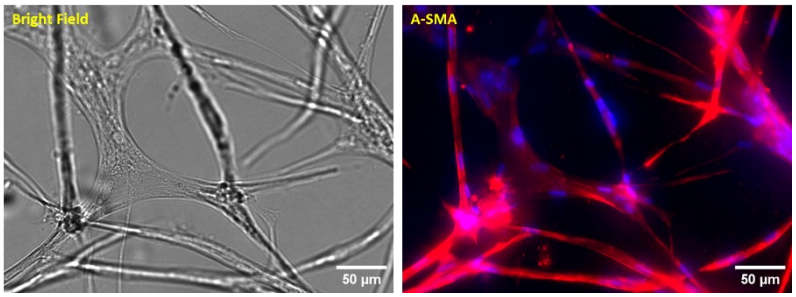

Figure 2. Representative field of view of a 3DProSeed StromaLine Lung CAF culture showing a bright-field image (one slice at the middle of the stack) and the corresponding immunofluorescence image (red) for α-SMA. Cell nuclei (blue) were counter-stained with Hoechst. The images were acquired under wide-field LED illumination with a Cytation1 BioTek imager at 20x magnification. Scale bar: 50 µm.

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Quality Control

The patient donor for the StromaLine Lung CAF (part nr. ECT.STRL.LUNCAF.096/01) was tested negative for HAV, HBV, HCV, HIV-1, and HIV-2 according to FDA regulations. Cell viability after recovery from cryopreservation has been assessed to > 80%. The cells have been tested negative for mycoplasma, bacteria, and fungi contamination.The StromaLine Lung CAF Medium (part nr. ECT.STRL.LUNCAF.096/02) was formulated for optimal growth of the StromaLine Lung CAF (part nr. ECT.STRL.LUNCAF.096/03) on the StromaLine Lung CAF Assay Plate.

The hydrogel formulation of the StromaLine Lung CAF Assay Plate (part nr. ECT.STRL.LUNCAF.096/03) was optimized for the growth of the StromaLine Lung CAF (part nr. ECT.STRL.LUNCAF.096/01). It was tested negative for microorganisms according to ISO 11737-1, and it has a particle count/well <10, based on microscopic inspection.

Certificates of analysis (CoA) for all products contained in the StromaLine Lung CAF system are available upon request.

Warranty

The products contained in the StromaLine Lung CAF system are performance assayed together and guaranteed to lead within 6-12 days to the development of α-SMA and FN positive cell cultures with a morphology and density as shown in Figure 1 in section 4, when following the protocol delineated in section 3.

This product is intended for research use only. It is not approved for human or veterinary use, for application to humans or animals, or for use in diagnostic or clinical or *in vitro* procedures.

Warning

STROMALINE LUNG CAF (ECT.STRL.LUNCAF.096/01) IS TESTED NEGATIVE FOR HBV, HCV, HIV-1, HIV-2 AND SYPHILIS ACCORDING TO FDA REGULATIONS, THIS MATERIALS SHOULD BE HANDLED AS POTENTIALLY BIOHAZARDOUS (BIOLOGICAL SAFETY LEVEL 2), FOLLOWING APPROPRIATE INSTITUTIONAL PROCEDURES AND UNVERSAL PRECAUTIONS.

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