3DProSeed™ StromaLine Melanoma Cancer-Associated Fibroblasts

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

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

Contents and storage

Product	Part number	Quantity	Storage	Description
StromaLine Melanoma CAF	ECT.STRL.MECAF.096/01	1x vial, 10^6 cells	Liquid N_2	Cryopreserved human malignant melanoma CAF
StromaLine Melanoma CAF Medium	ECT.STRL.MECAF.096/02	2x bottles 500 mL	2-4 °C	Low-serum culture medium optimized for the growth of the StromaLine Melanoma CAF
StromaLine Melanoma CAF 3DProSeed hydrogel Assay Plate	ECT.STRL.MECAF.096/03	1x96-well hydrogel plate	RT in correct orientation	3DProSeed 96-well glass-bottom hydrogel plate optimized for the growth of the StromaLine Melanoma CAF

Product Overview

The 3DProSeed™ StromaLine Melanoma Cancer-Associated Fibroblasts (CAF) is a pre-developed human stromal model made of patient-derived Melanoma 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 Melanoma 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 Melanoma CAF Assay Plate at room temperature and the StromaLine Melanoma CAF Medium at 37 °C for at least 30 min prior to use.
2. Thaw the frozen StromaLine Melanoma 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 Melanoma 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 50,000 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 Melanoma 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

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storage saline buffer. Do not touch or aspirate 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 Melanoma CAF Assay Plate. This will achieve a cell density of 10,000 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 Melanoma 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 Melanoma CAF system, as well as their ability to assemble a fibronectin (FN)-rich extracellular matrix is assessed by immunofluorescence (Figure 2).

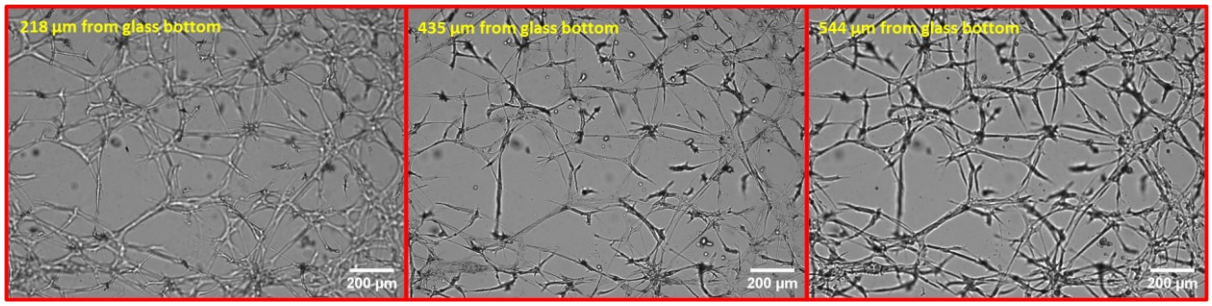

Figure 1. Representative field of view of the 3DProSeed StromaLine Melanoma CAF culture. Bright-field images were acquired at different focal planes (218, 435, and 544 µm from the glass bottom respectively), showing a 3D cellular network spanning a volume of ~300 µm. Such cell morphology and density can be achieved within 6-12 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.

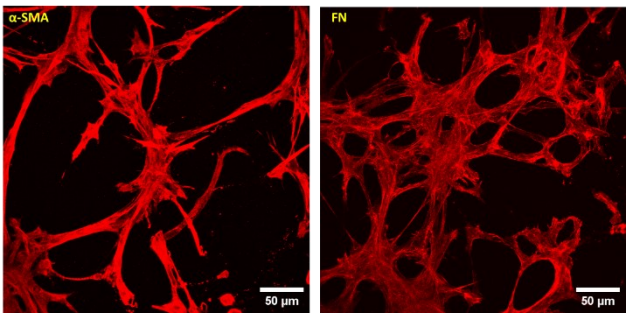

Figure 2. Representative immunofluorescence images for α-SMA and FN (red) of the 3DProSeed StromaLine Melanoma CAF culture. Cell nuclei (blue) were counter-stained with Hoechst. Fluorescence images were acquired with a Leica SP8 confocal microscope at 30x magnification using a long-distance objective, and maximum intensity z-projections are shown. Scale bar: 50 µm..

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Quality Control

The patient donor for the StromaLine Melanoma CAF (part nr. ECT.STRL.MECAF.096/01) was tested negative for HBV, HCV, HIV-1, and Syphilis according to FDA regulations. Cell viability after recovery from cryopreservation has been assessed to 86.2% by Propidium Iodide staining. The cells have been tested negative for mycoplasma, bacteria, and fungi contamination..

The StromaLine Melanoma CAF Medium (part nr. ECT.STRL.MECAF.096/02) was formulated for optimal growth of the StromaLine Melanoma CAF (part nr. ECT.STRL.MECAF.096/03) on the StromaLine Melanoma CAF Assay Plate.

The hydrogel formulation of the StromaLine Melanoma CAF Assay Plate (part nr. ECT.STRL.MECAF.096/03) was optimized for the growth of the StromaLine Melanoma CAF (part nr. ECT.STRL.MECAF.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 Melanoma CAF system are available upon request.

Warranty

The products contained in the StromaLine Melanoma 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 MELANOMA CAF (ECT.STRL.MECAF.096/01) IS TESTED NEGATIVE FOR HBV, HCV, HIV-1, 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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