3DProSeed™ StromaLine Bone-Marrow Vascular Niche

(Catalog Number: ECT.STRL.BMVAS.096c)

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

Contents and storage

Product	Part number	Quantity	Storage	Description
StromaLine Bone-Marrow MSC	ECT.STRL.MSC	1x vial, ≥0.75x10^6 cells	Liquid N_2	Cryopreserved multipotent stromal cells isolated from normal (non-diabetic) adult human bone marrow.
StromaLine Bone-Marrow EC	ECT.STRL.BMEC	1x vial, 10^6 cells	Liquid N_2	Cryopreserved endothelial cells isolated from the iliac crests of normal human donors; immortalized by transfection with the SV40 virus and stably expressing EGFP under the CMV promoter and puromycin as the selection marker
StromaLine Bone-Marrow MSC Medium	ECT.STRL.MSC_M	1x complete medium (500 mL)	2-4 °C	Culture medium optimized for the growth of the StromaLine Bone-Marrow MSC and Vascular Niche.
StromaLine Bone-Marrow EC Medium	ECT.STRL.BMEC_M	1x complete medium (500 mL)	2-4 °C	Culture medium optimized for the growth of the StromaLine Bone-Marrow Vascular Niche.
Stroma Line Assay Microtiter Plate	ECT.PSSTRL	1x96-well hydrogel plate	RT in correct orientation	96-well glass-bottom hydrogel plate optimized for the growth of the StromaLine Bone-Marrow Vascular Niche

Product Overview

The StromaLine Bone-Marrow Vascular Niche is an optimized platform for generating 3D co-cultures of mesenchymal stromal cells (MSC) and endothelial cells (EC) isolated from normal adult human bone marrow on a synthetic PEG-based hydrogel that allows the deposition and assembly of a native extracellular matrix resembling the stromal component of the bone-marrow vascular niche. The hydrogel is optically transparent, allowing a wide range of microscopy-based 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 hydrogel is offered pre-casted and ready to use in a 96-well plate format, and the cells can be delivered directly into your laboratory pre-plated, growing in the hydrogel plate or cryopreserved and ready for seeding in the plate.

The StromaLine cells, medium, and hydrogel plate are quality tested together and guaranteed to give optimum performance as a complete system.

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Seeding Protocol for Cryopreserved Cells

Establishment of the MSC supporting mono-culture

1. Thaw the StromaLine Bone-Marrow MSC Medium (delivered frozen) at 37 °C for upon arrival or prior to use. Do not refreeze. Prepare 50-mL aliquots and store at 4 °C for up to 1 month. Avoid repeating cycles of warming as this results in reduced activity of the growth factors used to supplement the medium and suboptimal culture growth.
2. Bring the StromaLine Assay Microtiter Plate at room temperature and the StromaLine Bone-Marrow MSC Medium aliquot to be used at 37 °C for at least 30 min prior to use.
3. Thaw the frozen StromaLine Bone-Marrow MSC vial directly upon arrival or after storing in liquid N_2 in a 37 °C water bath for a maximum of 90 sec.
4. Quickly transfer the thawed contents from the cell vial to a 50-mL conical tube containing 5 mL of StromaLine Bone-Marrow MSC Medium, pre-warmed at 37 °C. Centrifuge at 500xg for 5 min. Discard the supernatant and resuspend the cell pellet in 20 mL StromaLine Bone-Marrow MSC Medium. This step generates a cell suspension with a density of ~50,000 cells/mL, sufficient to seed an entire 96-well plate. We recommend this density for optimal results.
5. Carefully peel off the sealing adhesive foil from the StromaLine Assay Microtiter 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 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 remains in the well, it will not affect negatively culture development.
6. Add 200 µL/well of the cell suspension prepared in step 4 to the StromaLine Assay Microtiter 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% CO2 atmosphere. Change the medium after 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. However, we recommend proceeding with the sequential seeding of the StromaLine Bone-Marrow EC cells at day 5.

Establishment of the MSC-EC co-culture

7. Thaw the StromaLine Bone-Marrow EC Medium (delivered frozen) at 37 °C for upon arrival or prior to use. Do not refreeze. Prepare 50-mL aliquots and store at 4 °C for up to 1 month. Avoid repeating cycles of warming as this results in reduced activity of the growth factors used to supplement the medium and suboptimal culture growth.
8. Thaw the frozen StromaLine Bone-Marrow EC vial that has been stored in liquid N_2 in a 37 °C water bath for a maximum of 90 sec.
9. Quickly transfer the thawed contents from the cell vial to a 50-mL conical tube containing 5 mL of StromaLine Bone-Marrow EC Medium, pre-warmed at 37 °C. Centrifuge at 500xg for 5 min. Discard the supernatant and resuspend the cell pellet in 20 mL StromaLine Bone-Marrow EC Medium. This step generates a cell suspension with a density of 50,000 cells/mL, sufficient to seed an entire 96-well plate. We recommend this density for optimal results.
10. Take the StromaLine Assay Microtiter Plate with the growing MSC supporting culture from the 37 °C incubator just prior to EC seeding. 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 culture medium. Do not touch or aspirate right over the hydrogel to prevent damaging the growing culture. Avoid aspirating the storage buffer using a vacuum pump as the suction force may damage the hydrogel. If a small amount of the culture medium remains in the well, it will not affect negatively the subsequent co-culture development.
11. Add 200 µL/well of the cell suspension prepared in step 3 to the StromaLine Assay Microtiter Plate with the growing MSC supporting culture. 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% CO2 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 7 days.

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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 StromaLine Bone-Marrow Vascular Niche system relies on the sequential seeding of bone-marrow-derived MSC and EC on the StromaLine Assay Microtiter Plate to support the growth of an EC network mimicking the bone marrow vascular niche. The stable expression of EGFP in the StromaLine Bone-Marrow EC allows monitoring in real time the growth of the EC network on the supporting MSC culture. EC outgrowth from the initially formed cell clusters onto the spindle-like underlying MSC can be observed already 3 days after EC seeding, and a well-formed EC network is developed by day 7 (Figure 1).

The EC network extends in 3D, covering at least a volume of 200 µm in depth (Figure 2). No such EC network can be formed in the absence of the supporting MSC culture. Seeding EC alone in the StromaLine Assay Microtiter Plate, in the absence or presence of MSC-conditioned medium, results in spherical cell clusters, which show no signs of outgrowth and eventually die.

The StromaLine Bone-Marrow Vascular Niche co-culture system creates an extracellular microenvironment with features of the vascular basement membrane. In particular, secretion and deposition of fibronectin and laminin-5 is observed in close proximity to the EC network (Figure 3). Allowing the co-cultures to develop for longer periods of time (16 days) results in enhanced deposition of fibronectin but reduced amounts of laminin-5.

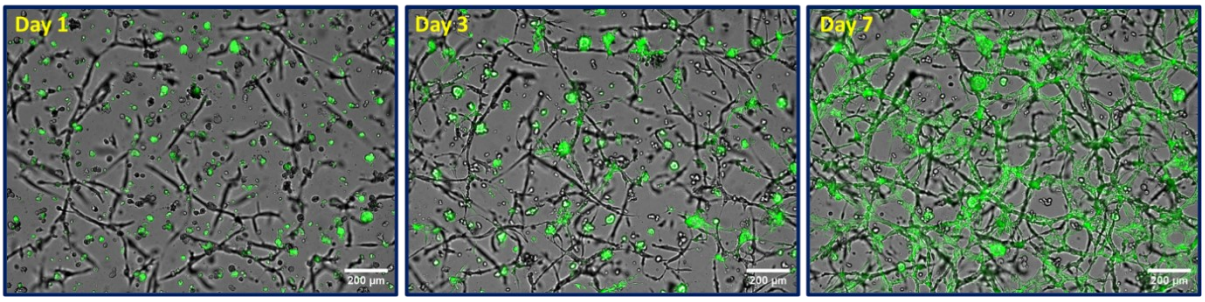

Figure 1. Time progression of the StromaLine Bone-Marrow Vascular Niche co-culture (bright field/GFP overlays). Bright-field and GFP images were acquired at days 1, 3, and 7 following the seeding of the EGFP-expressing StromaLine Bone-Marrow EC on StromaLine Bone-Marrow MSC cultures that were grown for 5 days on the StromaLine Assay Microtiter Plate. EC outgrowth onto the underlying MSC cultures can be observed already at day 3, while a well-developed network is formed by day 7. The images were acquired with a Cytation1 BioTek imager at 4x magnification. Scale bar: 200 µm.

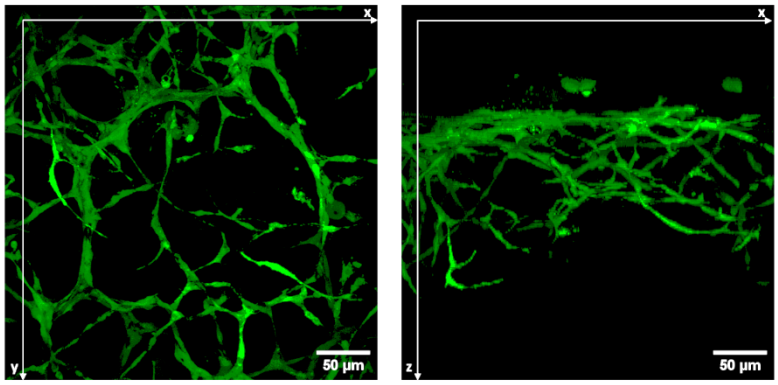

Figure 2. 3D projections (left panel: xy; right panel; xz) from a representative field of view of the EGFP-expressing EC network of the StromaLine Bone-Marrow Vascular Niche co-culture system (MSC culture: 5 days; subsequent EC culture: 7 days; total culture time: 12 days). Fluorescence images were acquired with an Olympus IXplore SpinSR10 confocal microscope at 30x magnification. Scale bar: 50 µm.

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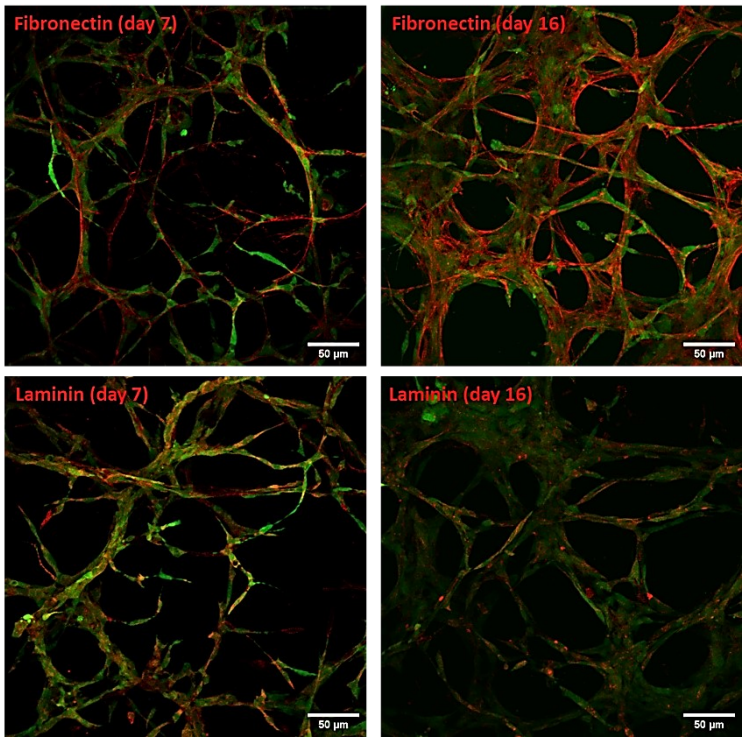

Figure 3. Representative immunofluorescence images for fibronectin and laminin-5 (red) of the StromaLine Bone-Marrow Vascular Niche co-culture system (MSC culture: 5 days; subsequent EC culture: 7 or 16 days; total culture time; 12 or 21 days respectively). Red fluorescence/GFP overlays are shown. Fluorescence images were acquired with an Olympus IXplore SpinSR10 confocal microscope at 30x magnification, and maximum intensity z-projections are shown. Scale bar: 50 µm.

Quality Control

The patient donor for the StromaLine Bone-Marrow MSC (ECT.STRL.MSC) has been tested negative for HBV, HCV, and HIV-1 according to FDA regulations. Cell viability after recovery from cryopreservation has been assessed at 76%. The cells have been tested negative for mycoplasma, bacteria, yeast, and fungi contamination.

The parental cell line, from which the EGFP-expressing StromaLine Bone-Marrow EC (ECT.STRL.BMEC) was derived, has been tested negative for HIV, HepB, HPV, EBV, and CMV according to FDA regulations. Cell viability after recovery from cryopreservation has been assessed at 85%. The cells have been tested negative for mycoplasma, bacteria, yeast, and fungi contamination. The species determination for the cell line has been done by STR analysis.

The StromaLine Bone-Marrow MSC Medium (ECT.STRL.MSC_M) has been formulated for optimal growth of the StromaLine Bone-Marrow MSC (ECT.STRL.MSC) on the StromaLine Assay Microtiter Plate (ECT.PSSTRL).

The hydrogel formulation of the StromaLine Assay Microtiter Plate (ECT.PSSTRL) has been optimized for the growth of the StromaLine Bone-Marrow MSC (ECT.STRL.MSC). It has been 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 Bone-Marrow MSC system (ECT.STRL.BMVAS.096c) are available upon request.

Warranty

The products contained in the StromaLine Bone-Marrow Vascular Niche system are performance assayed together and, when following the protocol delineated in section 3, are guaranteed to lead within 7 days to the development of an EC network with a morphology and density as shown in Figure 1 in section 4, associated with an extracellular matrix positive for fibronectin and laminin-5.

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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 clinical or in vitro diagnostic procedures.

Warning

WHILE THE PATIENT DONOR FOR THE STROMALINE BONE-MARROW MSC (ECT.STRL.MSC) HAS BEEN TESTED NEGATIVE FOR HBV, HCV, HIV-1, AND HIV-2, AND THE PARENTAL CELL LINE OF THE STROMALINE BONE-MARROW EC (ECT.STRL.BMEC) HAS BEEN TESTED NEGATIVE FOR HIV, HEPB, HPV, EBV, AND CMV, ACCORDING TO FDA REGULATIONS, THIS MATERIAL SHOULD BE HANDLED AS POTENTIALLY BIOHAZARDOUS (BIOLOGICAL SAFETY LEVEL 2), FOLLOWING APPROPRIATE INSTITUTIONAL PROCEDURES AND UNIVERSAL PRECAUTIONS.

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