# 3DProSeed™ StromaLine Bone-Marrow Mesenchymal Stromal Cells

(Catalog Number: ECT.STRL.BMMSC.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, $10^6$ cells | Liquid $N_2$ | Cryopreserved multipotent stromal cells isolated from normal (non-diabetic) adult human bone marrow. |
| 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. |
| 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 MSC. |

## Product Overview

The StromaLine Bone-Marrow Mesenchymal Stromal Cells (MSC) is an optimized platform for generating 3D cultures of multipotent stromal cells isolated from normal (non-diabetic) 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. 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.

### Seeding Protocol for Cryopreserved Cells

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 N2 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 the entire 96-well plate. We recommend this density for optimal results.
5. Carefully peel off the sealing adhesive foil from the StromaLine Assay Micortiter Plate. A liquid

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2022-V1

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 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 StromaLine Bone-Marrow MSC 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 alpha-smooth muscle actin (α-SMA) and of the MSC markers CD73 and CD90 in cells grown in StromaLine Bone-Marrow MSC system, as well as the assembly of a fibronectin (FN)-rich extracellular matrix, is assessed by immunofluorescence (Figure 2).

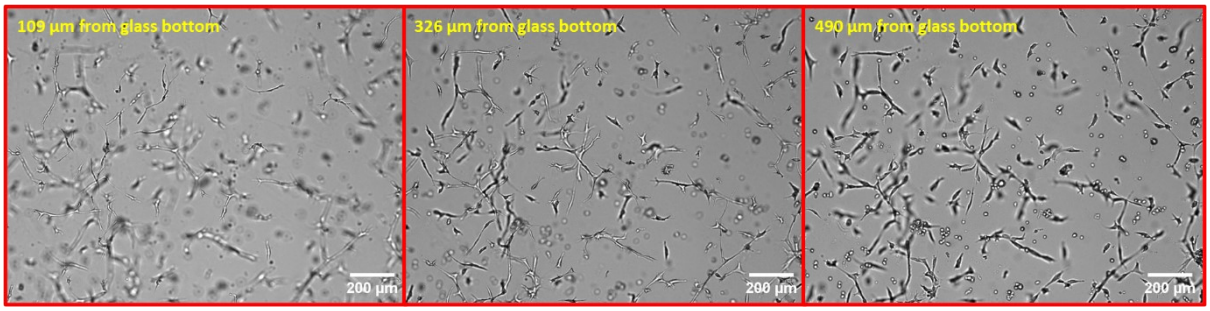


**Figure 1.** Representative field of view of the StromaLine Bone-Marrow MSC culture. Bright-field images were acquired at different focal planes (109, 326, and 490 µm from the glass bottom respectively), showing a 3D cellular network spanning a volume of ~400 µm. Such cell morphology and density can be achieved within 13 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.

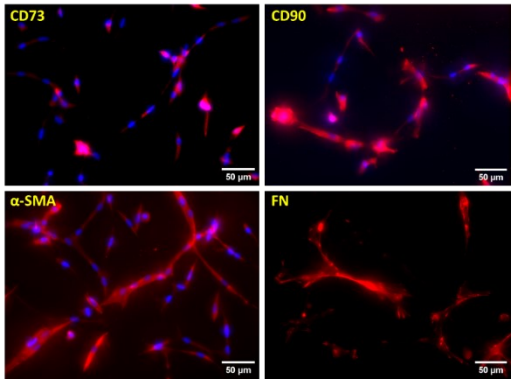


**Figure 2.** Representative immunofluorescence images for CD73, CD90, α-SMA, and FN (red) of the StromaLine Bone-Marrow MSC culture. Cell nuclei (blue) were counter-stained with Hoechst in the samples stained for the cellular markers CD73, CD90, and α-SMA. Fluorescence images were acquired under wide-field LED illumination with a Cytation1 BioTek imager at 20x magnification, and maximum intensity z-projections are shown. Scale bar: 50 µm.

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2022-V1

## 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 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.BMMSC.096c) are available upon request.

## Warranty

The products contained in the StromaLine Bone-Marrow MSC system are performance assayed together and, when following the protocol delineated in section 3, are guaranteed to lead within 10 days to the development of CD73, CD90, α-SMA, and FN positive cell cultures with a morphology and density as shown in Figure 1 in section 4. Note that the StromaLine Bone Marrow MSC are a heterogeneous population with not all cells present expressing the MSC marker CD73 (Figure 2).

**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 diagnostics procedures.

## Warning

WHILE THE PATIENT DONOR FOR THE STROMALINE BONE-MARROW MSC (ECT.STRL.MSC) IS TESTED NEGATIVE FOR HBV, HCV, HIV-1, AND HIV-2 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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