Human Adipose Mesenchymal Stem Cell Kit

Product Manual for the following Cat. Nos.
SCR038
SCC038

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

USA & Canada Phone: +1(800) 645-5476
In Europe, please contact Customer Service:
France: 0825.045.645;
Spain: 901.516.645 Option 1
Germany: 01805.045.645
Italy: 848.845.645
United Kingdom: 0870.900.46.45
For other locations across the world please visit www.millipore.com/offices

Introduction

Mesenchymal stem cells (MSCs) are non-hematopoietic cells with multi-lineage potential that hold great promise for regenerative medicine. In defined *in vitro* assays, mesenchymal stem cells have been shown to readily differentiate into lineage-specific cells that form bone, cartilage, fat, tendon, and muscle tissues (1, 2). Adipose tissue represents an abundant, accessible source of multipotent adipose-derived stromal cells.

EMD Millipore's Human Adipose MSCs are isolated from human adipose tissue and are cryopreserved at p2 to ensure optimal phenotype and the highest viability and plating efficiency. Each lot of human adipose MSCs originates from a single donor of human lipoaspirate tissue. These cells proliferate as an adherent cell monolayer and have shown a strong capacity for expansion *in vitro* while maintaining their potential for differentiation to multiple lineages including adipocytes, osteoblasts and chondrocytes. EMD Millipore's Human Adipose MSCs uniformly express appropriate MSC markers including the integrin CD29, matrix receptors CD44, CD105 and stromal cell-associated markers CD73, CD90, and CD166. The cells are negative for hematopoietic lineage markers CD14, CD31, CD34, and CD45.

The following products are recommended for use with the Human Adipose MSCs:

For optimal cell growth and expansion: please use the Human Mesenchymal-LS Expansion Medium (Cat. No. SCM023).

For differentiation to adipocytes: please use the Mesenchymal Stem Cell Adipogenesis Kit (Cat. No. SCR020).

For differentiation to osteocytes: please use the OsteoMAX-XF™ Differentiation Medium (Cat. No. SCM121).

For immunocytochemical characterization: please use the Human Mesenchymal Stem Cell Characterization Kit (Cat. No. SCR067) along with additional antibodies including anti-Endoglin (CD105, Cat. No. MABT117) and CD73 (Cat. No. MABD122).

For flow analysis: please use the FlowCellect™ Human Mesenchymal Stem Cell Characterization Kit (Cat. No. FCSC100184).

For Research Use Only; Not for use in diagnostic procedures

Materials Provided

1. >1 x 10^6 viable Human Adipose Mesenchymal Stem Cells: (Cat. No. SCC038) derived from adult human lipoaspirate and are cryopreserved as secondary cells. Store in liquid nitrogen.

2. 500 mL Human Mesenchymal-LS Expansion Medium: (Cat. No. SCM023):

   - Human Mesenchymal-LS Basal Medium (Part. No. SCMM-BM) One 480 mL bottle. Store at 2 – 8°C.
   - Human Mesenchymal-LS Supplement Kit (Part No. SCMM002-S). Store at -20°C.

Materials Required But Not Provided

1. Tissue culture-wares and supplies
2. Accutase (Cat. No. SCR005)
3. Phosphate-Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
4. OsteoMAX-XF™ Differentiation Medium (Cat. No. SCM121)
5. Mesenchymal Stem Cell Adipogenesis Kit (Cat. No. SCR020)
6. Human Mesenchymal Stem Cell Characterization Kit (Cat. No. SCR067)
7. FlowCellect™ Human Mesenchymal Stem Cell Characterization Kit (Cat. No. FCSC100184)
8. Characterization Antibodies: CD105 (Cat. No. MABT117), CD90 (Cat. No. CBL415), CD44 (Cat. No. CBL154), CD73 (Cat. No. MABD122), STRO-1 (Cat. No. MAB4315), CD14 (Cat. No. MAB1219), CD19 (Cat. No. MAB1794)
9. Trypan Blue
10. Millicell EZ SLIDE 8-well glass, sterile (Cat. No. PEZGS0896)
11. Hemocytometer
12. Microscope with appropriate fluorescent filters.

Characterization of Cells

EMD Millipore's Human adipose mesenchymal stem cells are derived from human adipose tissue and have been validated for high expression level of cell surface molecules that are present on mesenchymal stem cells: CD44, CD105, CD73, CD90, CD166, and for their absence of hematopoietic lineage markers, CD14, CD31, CD34, and CD45. The cells have also been validated for their self-renewal and multi-lineage differentiation capacities (please refer to product manual figures for representative data). Cells display normal karyotype as assessed by G-banding of 20 metaphase cells and tested negative for mycoplasma and for bacterial and fungal growth. Cells are also negative for HIV-1, HIV-2, HBV, and HCV as assessed by PCR analysis.

Storage/Handling

Human Adipose Mesenchymal Stem Cells: Cat. No. SCC038) should be stored in liquid nitrogen. The cells can be passaged for at least three passages (or ten population doublings) without significantly affecting the cell surface marker expression and differentiation potential.

Human Mesenchymal-LS Expansion Medium (Cat. No. SCM023). Each media kit consists of two components:

- Human Mesenchymal-LS Basal Medium (Part. No. SCMM-BM) One 480 mL bottle. Store at 2 – 8°C. The special UV protective packaging helps protect the basal medium from light damage, however users should take care to protect basal medium from extended exposure to light.

- Human Mesenchymal-LS Supplement Kit (Part No. SCMM002-S) Store at -20°C.

Do not use product beyond expiration date. All components are guaranteed stable until the expiration date stated on the individual labels.

Thawing of Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue culture ware surfaces without any additional coating.

2. Remove the vial of Human Mesenchymal Stem Cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**

3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.

4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.

5. Using a 10 mL pipette, slowly add dropwise 9 mL Human Mesenchymal-LS Expansion Medium (Cat. No. SCM023) (pre-warmed to 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock.**

6. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT: Do not vortex the cells**.

7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.

8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).

9. Resuspend the cells in a total volume of 10 mL of Human Mesenchymal-LS Expansion Medium (Cat. No. SCM023) (pre-warmed to 37°C).

10. Plate the cell mixture onto 10-cm tissue culture plates or multiple T75 tissue culture flasks. The recommended plating density is 5000 cells/cm^2 (approximately equivalent to plating cells recovered from one cryovial into three 10-cm tissue culture plates or three T75 tissue culture flasks).

11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

12. The next day, exchange the medium with fresh Human Mesenchymal-LS Expansion Medium (pre-warmed to 37°C). Exchange with fresh medium every two to three days thereafter.

13. When the cells are approximately 80% confluent (3-4 days after plating cells at the density around 5000 cells/cm^2), they can be dissociated with Accutase (Catalog No. SCR005) and passaged or alternatively frozen for later use.

Subculturing

1. Carefully remove the medium from the 10-cm tissue culture plate or T75 tissue culture flask containing the confluent layer of human mesenchymal stem cells.

2. Apply 3-5 mL of Accutase Solution and incubate in a 37°C incubator for 3-5 minutes.

3. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.

4. Add 8 mL of Human Mesenchymal-LS Expansion Medium (Cat. No. SCM023, pre-warmed to 37°C) to the plate.

5. Gently rotate the plate to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.

6. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.

7. Discard the supernatant.

8. Apply 2 mL of Human Mesenchymal-LS Expansion Medium (Cat. No. SCM023, pre-warmed to 37°C) to the conical tube and resuspend the cells thoroughly. **IMPORTANT: Do not vortex the cells.**

9. Count the number of cells using a hemocytometer.

10. Plate the cells to the desired density into the appropriate flasks, plates, or wells in Human Mesenchymal-LS Expansion Medium. The recommended plating density is 5000 cells/cm^2.

Characterization of Human Adipose Mesenchymal Stem Cells (Cat. No. SCC038)

The following are representative results obtained using the Human Adipose Mesenchymal Stem Cells (Cat. No. SCC038).

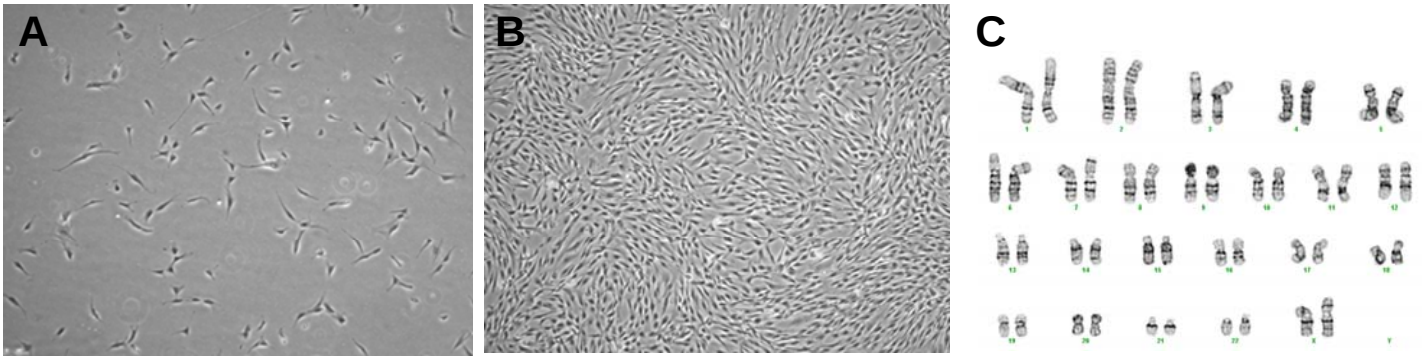

Figure 1. Phase contrast images of Human Adipose Mesenchymal Stem Cells (Catalog No. SCC038) one (**A**) and three (**B**) days after thawing. Cells were plated at 5000 cells/cm^2. Cells are ≥ 80% confluent after 3-4 days culture and are ready to be passaged (**B**). Cells possess an apparently normal karyotype (C). Cytogenetic analysis was performed by Cell Line Genetics on twenty G-banded metaphase cells. All twenty cells demonstrated an apparently normal female karyotype (46, XX). No abnormal cells were detected.

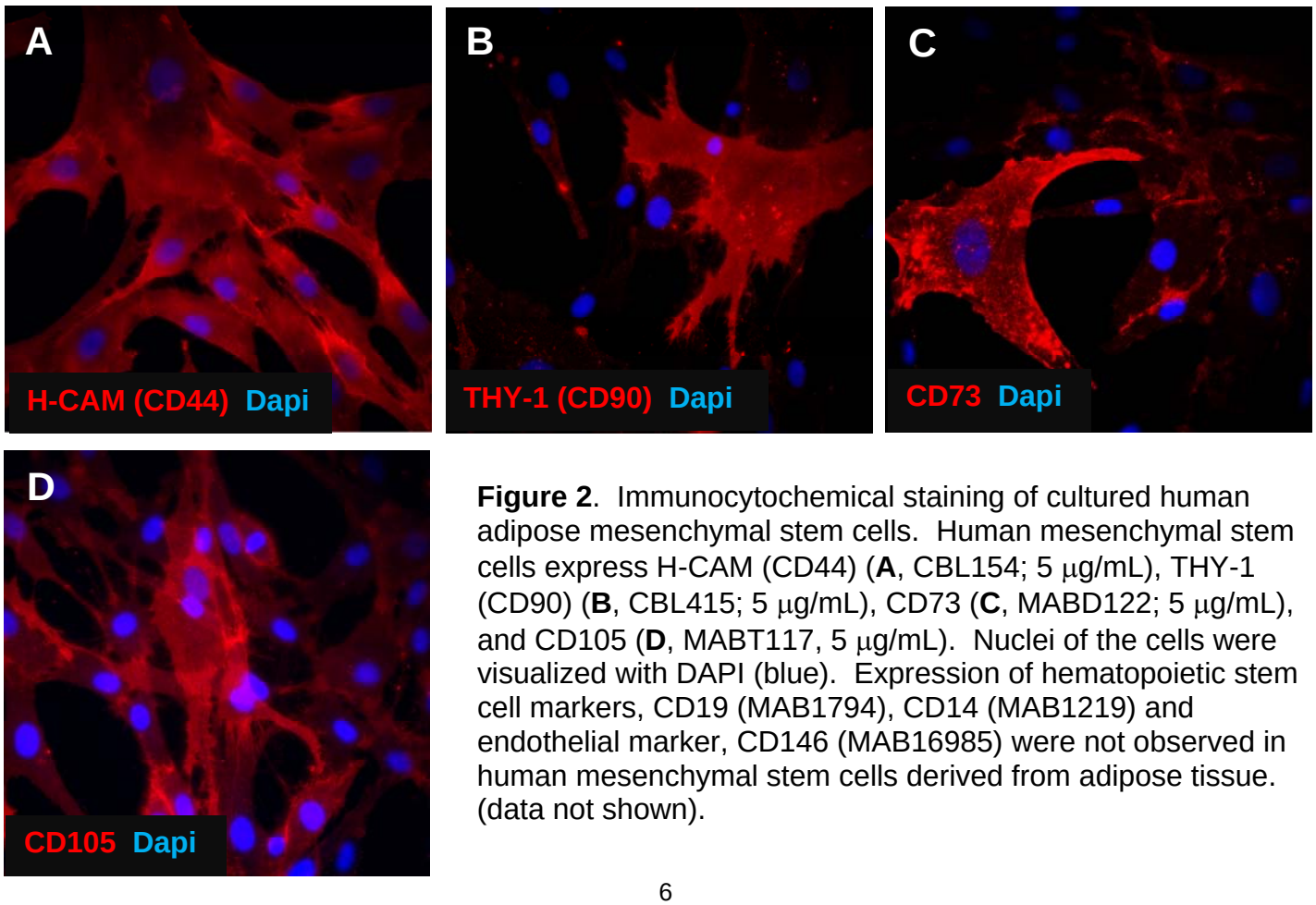

Figure 2. Immunocytochemical staining of cultured human adipose mesenchymal stem cells. Human mesenchymal stem cells express H-CAM (CD44) (**A**, CBL154; 5 μg/mL), THY-1 (CD90) (**B**, CBL415; 5 μg/mL), CD73 (**C**, MABD122; 5 μg/mL), and CD105 (**D**, MABT117, 5 μg/mL). Nuclei of the cells were visualized with DAPI (blue). Expression of hematopoietic stem cell markers, CD19 (MAB1794), CD14 (MAB1219) and endothelial marker, CD146 (MAB16985) were not observed in human mesenchymal stem cells derived from adipose tissue. (data not shown).

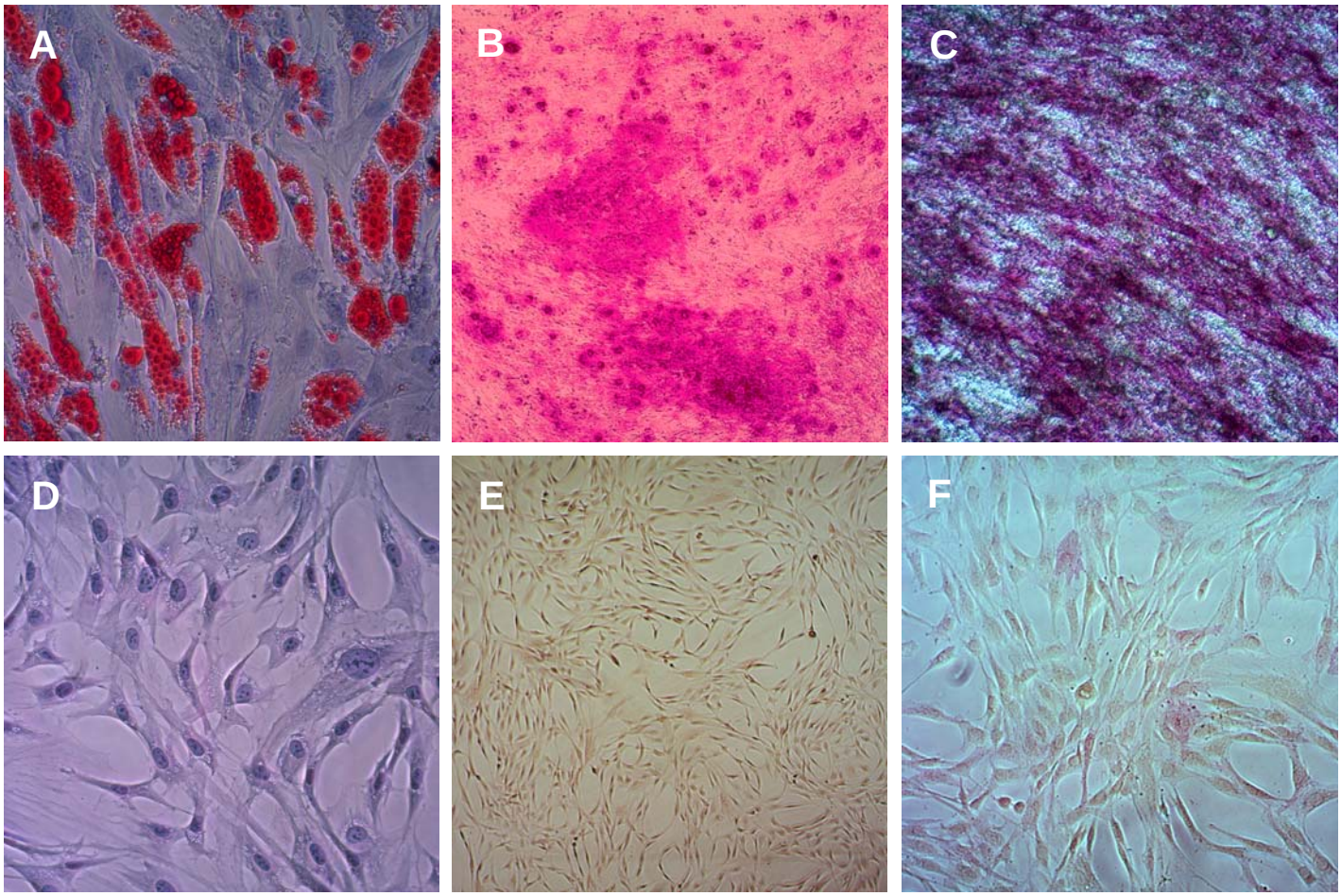

Figure 3. Human Adipose Mesenchymal Stem Cells (Cat. No. SCC038) are multipotent. Human adipose mesenchymal stem cells were differentiated in adipogenic (**A**) and osteogenic (**B, C**) differentiation medium. Using EMD Millipore's Mesenchymal Stem Cell Adipogenesis Kit (Cat. No. SCR020), human adipose mesenchymal stem cells differentiated after 21 days to mature adipocytes as indicated by the accumulation of lipid vacuoles that stain with Oil Red O (**A**, 10X magnification). Control untreated human mesenchymal stem cells did not contain any lipid droplets (**D**). Using EMD Millipore's OsteoMAX-XF™ Differentiation Medium (Cat. No. SCM121), human mesenchymal stem cells readily differentiated to an ostoecyte lineage as indicated by Alizarin Red S (ARS) (**B**, 4X magnification) and alkaline phosphatase (**C**, 10X magnification) staining. Alizarin Red S staining demonstrates mineral deposition throughout the culture. Control untreated human mesenchymal stem cells did not stain for ARS (**E**) or alkaline phosphatase (**F**).

Interpretation of Results

The determination that a cell is or is not a mesenchymal stem cell is based on the differential expression of a panel of markers and cannot be determined based on the expression (or lack thereof) of a single marker. While certain markers may be expressed by many cell types, it is the concomitant expression of multiple markers by a single cell and the non-expression of others that ultimately identify the cell as a particular cell type. It is generally accepted that cells that express CD44, CD90, and STRO-1 but do not express CD14 and CD19 represent a mesenchymal stem cell population (2). Antibodies directed against CD44, CD90, and STRO-1 are provided as Mesenchymal Stem Cell positive selection markers. Mesenchymal Stem Cells will express each of these antigens and identification of a population of cells as Mesenchymal Stem Cells requires that the cells stain with each of the positive selection antibodies. In addition, antibodies to CD14 and CD19 are two surface markers that are present on leukocytes and B lymphocytes, respectively and are not expressed on mesenchymal stem cells. The presence of positive staining with either one of these negative selection markers in the mesenchymal stem cell population indicates contamination of the particular cell lineage in question.

Related Products

The following products are available from EMD Millipore as separate items:

1. Mouse anti-Human CD44, 100 µg (Cat. No. CBL154)
2. Mouse anti-Human THY-1, 100 µg (Cat. No. CBL415)
3. Mouse anti-STRO-1, 100 µL (Cat. No. MAB4315)
4. Mouse anti-Endothelial Cells (CD146), 100 µg (Cat. No. MAB16985)
5. Mouse anti-Human CD14, 100 µg (Cat. No. MAB1219)
6. Mouse anti Human B Cells (CD19), 100 µg (Cat. No. MAB1794)
7. Human Mesenchymal-LS Expansion Media Kit, 500 mL (Cat. No. SCM023)
8. Human Mesenchymal Stem Cell Characterization Kit (Cat. No. SCR067)
9. FlowCellect™ Human Mesenchymal Stem Cell Characterization Kit (Cat. No. FCSC100184)
10. Mesenchymal Stem Cell Adipogenesis Kit (Cat. No. SCR020)
11. OsteoMAX-XF™ Differentiation Medium (Cat. No. SCM121)
12. Human Mesenchymal Stem Cell Kit (Derived from Bone-Marrow) (Cat. No. SCR108)
13. Human Mesenchymal Stem Cells (Derived from hES Cells) (Cat. No. SCC036)

References

1. Prockop, D. J. (1997). Marrow stromal cells as stem cells for non-hematopoietic tissues. *Science* **276**: 71-74.

2. Pittenger, M. F., and Marshak, D. R. in *Stem Cell Biology* (Eds Marshak, D., R., Gardner, R. L., & Gottlieb, D.) (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).

Warranty

EMD Millipore Corporation ("EMD Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **EMD MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of EMD Millipore products appearing in EMD Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of EMD Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, EMD Millipore Corporation's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies EMD Millipore Corporation promptly of any such breach. If after exercising reasonable efforts, EMD Millipore Corporation is unable to repair or replace the product or part, then EMD Millipore shall refund to the Company all monies paid for such applicable Product. **EMD MILLIPORE CORPORATION SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), 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 or animals.