denovoMATRIX
ENABLING BIOLOGY IN VITRO

denovoCELLS

PREMIUM CELL LINE PRODUCTS

User guide

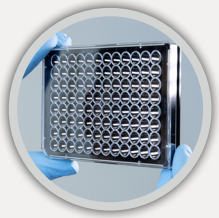
MESENCHYMAL STROMAL CELLS

BIOMATRICES FOR CELL CULTURE

screenMATRIX

A simple starting tool to find the best environment for any cell type.

- Available formats: 96-well plate
- Cat. No: S1001



isoMATRIX

A simple starting tool to find the best environment for any cell type.

- Available formats: T-flasks and well plates
- Cat. No: CXX04

myMATRIX MSC

Xeno/serum-free and chemically defined mesenchymal stromal cell culture.

- Available formats: T-flasks and well plates
- Cat. No: CXX01

myMATRIX iPSC

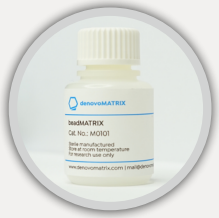
Xeno-free and chemically defined pluripotent stem cell culture.

- Available formats: T-flasks and well plates
- Cat. No: CXX05

beadMATRIX

Xeno-free and chemically defined 3D culture of mesenchymal stromal cells and iPSCs.

- Available formats: ready-to-use beads
- beadMATRIX Cat. No: M0101; beadMATRIX+ Cat. No: M0105



User guide

Human mesenchymal stromal cells (hMSCs) are a heterogeneous population of tissue-specific stem/progenitor cells that possess the ability to recreate the tissue from which they are isolated. They have been isolated from a wide range of adult and perinatal tissues with similar morphologies and characteristics. hMSCs show a fibroblast-like morphology and are capable of differentiating into adipogenic, osteogenic, and chondrogenic lineages. A specific subset of cell surface markers (CD markers) was defined by the International Society for Cell and Gene Therapy for the characterization of hMSCs: expression of CD73, CD105, and CD90 in at least 95% of the cell population and absence of the hematopoietic markers CD45, CD34, CD11b, or CD14.

denovoCELLS hMSCs are isolated from human bone marrow mononuclear cells using the isoMATRIX in combination with TheraPEAK™MSCGM™ Mesenchymal Stem Cell Growth Medium (Lonza). After expansion in the same medium on myMATRIX MSC to a population doubling level (PDL) of 4-6, cells were cryopreserved in CryoStorR CS10 (STEMCELL Technologies). The unique alliance of GMP-produced, serum-free expansion and cryopreservation medium and chemically defined biomatrices enables the manufacturing of hMSCs with high proliferation and differentiation potential.

QUALITY STATEMENT

Each lot of mesenchymal stromal cells is thoroughly tested for multiple parameters to fulfill our stringent quality control:

- The source material for the isolation of denovoCELLS hMSCS has been tested for the absence of HIV-1/2, HBV, and HCV.
- The isolated cells are further checked for cell morphology, proliferation potential, and viability.
- In addition, they are analyzed by flow cytometry for a panel of cell surface markers: CD73/90/105/44 and CD11b/14/34/45.
- We also test their differentiation capacity by inducing adipogenic, osteogenic, and chondrogenic differentiation.
- Finally, each lot is checked for microbial contaminants (fungi, yeast, bacteria, and mycoplasma).

INTENDED USE

denovoCELLS hMSCs are for in vitro research use only.

Not approved for human or veterinary use, for application to humans or animals, or for use in in vitro diagnostic or clinical procedures.

STORAGE

Upon arrival, check all containers for leakage or breakage. Cryopreserved cells should be placed directly into liquid nitrogen storage or seeded. directly.

Thawing

MEDIA CHOICE

We recommend using TheraPEAK™ MSCGM™Mesenchymal Stem Cell Growth Medium from Lonza for expansion of denovoCELLS hMSCs (#HBMMSC_04015151). TheraPEAK™ MSCGM™ is a serum-free medium composed of a basal medium and a corresponding supplement. Please prepare the medium and store it according to the manufacturer's instruction manual.

THAWING PREPARATION

- The recommended seeding density for hMSCs is 3,000 – 6,000 cells per cm^2. To set up cultures, calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used. myMATRIX MSC products are ready-to-use and do not require any processing prior to use. Label each flask with the passage number, strain number, cell type, and date.
- Add the appropriate amount of medium to the vessels (0.2-0.4 mL per cm^2) and allow them to equilibrate in a 37°C, 5% CO_2 humidified incubator for 30 min.
- In addition, prepare a centrifuge tube with 5 mL of pre-warmed medium.

THAWING CELLS TO START YOUR CULTURE

- Remove the cryovial from the liquid nitrogen container and immediately place on dry ice.
- Wipe the cryovial of cells with 70% ethanol before opening.
- In a biosafety cabinet, briefly twist the cap a quarter turn to relieve pressure, and then retighten.
- Quickly thaw the cryovial in a 37°C water bath for about 1-2 min. Do not submerge the vial completely.
- Closely watch the cryovial. Remove the vial and process further when a small amount of the ice is still visible.
- Wipe cryovial of cells with 70% ethanol and wipe dry to remove excess.
- Transfer into a biosafety cabinet and open the vial.

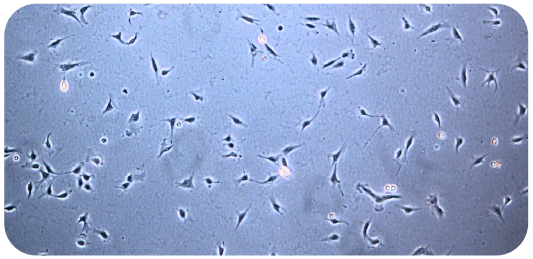


Figure 1. denovoCELLS hMSCs after thawing.

- Gently pipette the thawed cell suspension into the 5 ml of pre-warmed medium in the centrifuge tube.
- Centrifuge at 250-300 g for 5 minutes at room temperature.
- Resuspend the pellet in 1 ml of complete medium by gently pipetting up and down.
- Count the total number of viable cells.
- Add the calculated volume of cell suspension to each prepared flask and gently rock to disperse the suspension over the culture surface.
- Place the vessels in a humidified incubator at 37°C, 5% CO_2 for cell attachment and growth.

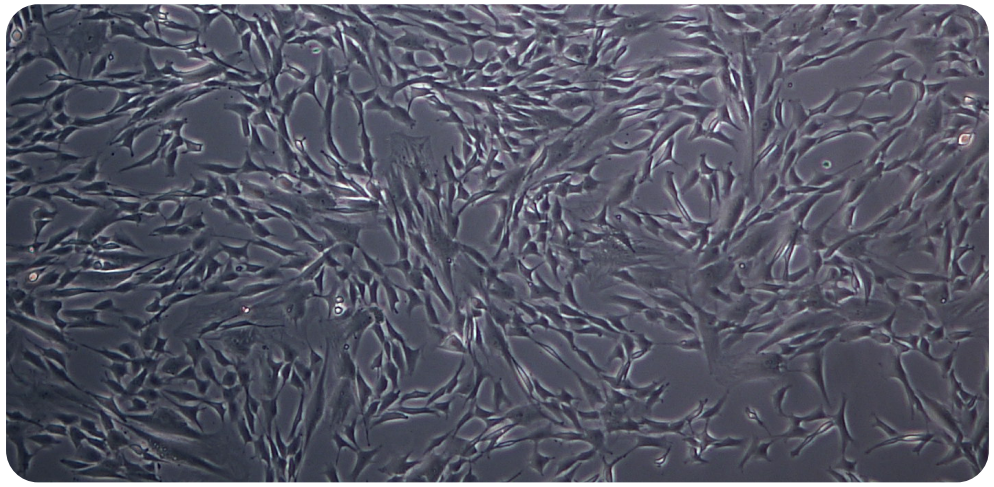


Figure 2. Expected morphology at 80-90% confluency.

What morphology can indicate:

- Small, spindle-shaped MSCs with shiny edges are indicative of rapid self renewal
- Increase in cell size are first signs of senescence
- MSC morphology in serum-free media is substantially different than in serum, with serum morphology resembling a senescent phenotype

Maintenance

GENERAL CONSIDERATIONS

hMSCs should be fed every 3-4 days. To feed the cultures, gently and completely remove the medium from the culture vessel and replace it with an equal volume of pre-warmed fresh medium. Return the culture vessels to the incubator.

SPLITTING PREPARATION

- We recommend using TheraPEAK™ MSCGM™Mesenchymal Stem Cell Growth Medium from Lonza for expansion of denovoCELLS hMSCs (#HBMMSC_04015151). TheraPEAK™ MSCGM™ is a serum-free medium composed of a basal medium and a corresponding supplement. Please prepare the medium and store it according to the instruction manual of the manufacturer.
- The basal medium (TheraPEAK™ MSCBM™) must be stored at 4°C to 8°C and the supplement (2 x 5 ml vials) in the -20°C freezer (non-self-defrosting).
- We also recommend using TrypLE™ Express Enzyme from Gibco™ for the dissociation of the denovoCELLS hMSCs. TrypLE™ Express Enzyme is an animal-origin-free recombinant enzyme alternative to porcine or bovine trypsin for the dissociation of attachment-dependent cell lines from plasticware. It cleaves peptide bonds on the C-terminal side of lysine and arginine but with greater specificity than native trypsin preparations due to the superior purity of TrypLE™. TrypLE™ Express Enzyme is formulated in DPBS/1 mM EDTA and is stable at room temperature.
- Calculate the number of vessels needed based on the recommended seeding density (3,000 – 6,000 cells per cm^2) and the surface area of the vessels being used. myMATRIX MSC products are ready-to-use and do not require any processing prior to use. Label each flask with the passage number, strain number, cell type, and date.

SUBCULTURING/SPLITTING

- Carefully aspirate and discard the medium from the culture vessels.
- Wash the attached cell layer with 1x phosphate-buffered saline (PBS) free of calcium and magnesium.
- Do not add the PBS directly on top of the cells but to one side of the vessel to prevent the disruption of the layer.
- Carefully aspirate and discard the wash solution.
- Add a sufficient volume of TrypLE™ Express Enzyme (Gibco™) to cover the cell layer (0.04-0.07 mL per cm^2).
- Incubate at 37°C in the incubator until cells have detached (max. 5-10 min). Observe cell monolayer using an inverted microscope to ensure complete cell detachment from the surface of the flask. Gently tap the flask to dislodge the cells if necessary.
- Once ≥90% of the cells are rounded and detached, add pre-warmed complete medium to the flask, at a minimum of the same volume as TrypLE™. No inactivation of TrypLE™ is required, dilution alone inactivates TrypLE™.
- Tilt flask in all directions to thoroughly rinse it.
- Carefully aspirate the cell suspension and transfer it to a centrifuge tube.
- Centrifuge at 250-300 g for 5 minutes at room temperature.
- Discard the supernatant and resuspend the pellet in 1 ml of complete medium by gently pipetting up and down.
- Count the total number of viable cells.
- Add the calculated volume of cell suspension and the appropriate volume of pre-warmed medium to each prepared flask and gently rock to disperse the suspension over the culture surface.
- Place the vessels in a humidified incubator at 37°C, 5% CO_2 for cell attachment and growth.

FREQUENTLY ASKED QUESTIONS

My hMSCs do not grow as expected. What could be the reason?
There are multiple reason that could negatively impact the growth performance of hMSCs. First, check your starting material.

- **Have the cells been stored correctly?**
  Make sure to immediately store your cells in liquid nitrogen after arrival without allowing the vial to begin to thaw.
- **Did you thaw your cells quickly and not too long after taking them out of the liquid nitrogen?**
  Thawing the cells only takes 1-2 min in a 37°C water bath. Stop the process when you see a small icy core remaining.
- **Did they show a high viability after thawing?**
  Your cells should show a viability of ≥ 80% after the thawing procedure.
- **Did your cells attach well?**
  Attachment of the cells is the first step for an efficient cell proliferation. Check your cells 24 hours after seeding. If the cells attached well they show a small, fibroblast-like morphology (Fig. 2, 3A). Cells with impaired attachment are shown in Fig. 3D.
- **Did you maintain your cells correctly?**
  The medium of hMSCs should be renewed every 3-4 days to ensure a proper nutrient concentration and to prevent the buildup of high levels of waste products. Measuring the levels of glucose and lactate in your culture medium can help you to judge the right time point for a medium change.
- **If you have cultured the cells before, did you harvest them at the right time point?** You should harvest your cells when they are 80-90% confluent (Fig. 3A). Letting them grow too confluent (Fig. 3B) will impair their proliferation and differentiation potential over time and induce senescence.
- **Are your cells already in senescence? How do I identify senescence?**
  First, check the morphology of your cells. Your cells will increase in size and start showing a flattened, extended morphology (Fig. 3C) when entering senescence. In addition, calculate the doubling time of your cell population. hMSCs will slow down their proliferative speed over time, which means their doubling time will increase.

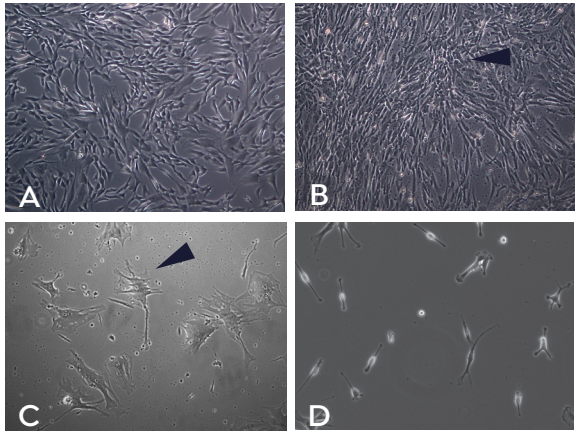


Figure 3. Morphology of MSCs. A) Healthy morphology of MSCs in serum-free media, B) Overgrown plate with MSCs, C) Senescent MSCs with enlarged morphology, D) Low attachment of MSCs after thawing.

Troubleshooting

How to calculate the doubling time?

$$doubling\ time = \frac{\ln(2)}{\text{growth rate}}$$

$$growth\ rate = \frac{\ln\left(\frac{N(t)}{N(0)}\right)}{t}$$

N(t) = number of cells at time of harvest
N(0) = number of seeded cells
t = time in culture (hours)

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