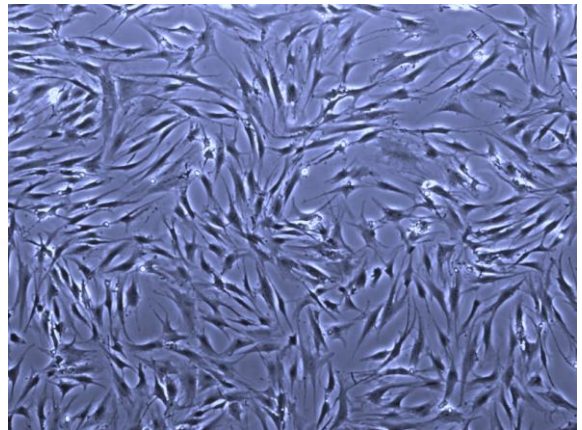


## KIT Human Mesenchymal Stem Cells

### Product Description

Human mesenchymal stem cells (hMSC) are isolated from human bone marrow. hMSC can be expanded and after induction they are able to differentiate into a number of different cell types, such as adipocytes, neuronal cells, chondrocytes or osteocytes.

hMSC are typically offered cryopreserved, 500 000 viable cells/vial at passage 2-3. hMSC are quality checked, the donors are negative for the virus species HIV (I, II or III) HBV and HCV.



For further subcultivation of hMSC we recommend to use the enclosed Collagen Cell Carrier, a compact membrane of pure, non-cross-linked bovine collagen type I, designed for the cultivation of cells on its surface. The Collagen Cell Carrier exhibits high mechanical stability and minimal autofluorescence and is sterilized by gamma radiation. These properties make it an excellent cell carrier for research applications and in regenerative medicine.

### Applications

hMSC are characterized by high proliferation rates and differentiation potential. They can be used in a great number of applications, such as research into mechanism or treatment of bone related conditions (e.g. osteoporosis) or toxicity testing of drugs in the preclinical phase. Since hMSC can be differentiated into adipocytes, these cells can be used for studying obesity. A large number of substances have been tested on hMSC, such as immunosuppressive agents, chemotherapeutic and radio-protective compounds.

### *Passaging*

For cell passaging or preparation of cell suspensions standard trypsinization procedures can be used to detach adherent hMSC from the surface of the vessel.

### **Storage:**

If you do not use the cells immediately, cells have to be stored at temperatures below -150°C (e.g. in gas phase of liquid nitrogen storage tank).

**Please note: CELLS ARE INTENDED FOR IN VITRO RESEARCH USE ONLY! NOT FOR CLINICAL DIAGNOSTIC OR THERAPEUTIC PROCEDURES. DO NOT USE IN HUMANS.**

**Since this material is of human origin, these cells have to be treated as potentially biologically hazardous material, even if serological tests are negative. S2 (Biological Safety Level 2) handling using adequate safety control methods and using suitable protective equipment is strongly recommended.**

### **BEFORE STARTING, PLEASE NOTE:**

Use appropriate cell culture-treated plates, hMSC growth medium, and reagents as well as aseptic techniques and ensure adequate growth environments.

Once Growth Medium has been supplemented, it has to be stored at 2 to 8°C. Pre-warm at least to room temperature before adding to any cell culture.

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### Prepare the Growth Medium

1. Warm the hMSC Basal Medium and the Supplements to 37°C
2. Add the Supplements directly to the Basal Medium
3. Use the Growth Medium for cell cultivation pre warmed at 37°C

*The Growth Medium can be stored at 4°C up to 6 weeks!*

### Prepare the Cells

1. Thaw the vial of frozen cells in a 37°C water bath with moderate agitation until a small amount of ice is left in the vial (approx. 1,5 to 2 minutes)
2. Transfer the vial to a laminar flow hood
3. Immediately sterilize the outside of the vial with isopropanol or ethanol (70 %)
4. Carefully transfer the cell suspension into a 15 mL tube
5. Rinse the vial with 1 mL warmed Growth Medium to recover remaining cells
6. Bring up the volume slowly by adding 10 mL of Growth Medium dropwise into the vial; swirl gently from time to time during this procedure
7. Centrifuge the cell suspension at 200 x g at room temperature for 5 minutes
8. Discard the supernatant and gently re-suspend the pellet in 1 to 2 mL Growth Medium
9. Count the cells

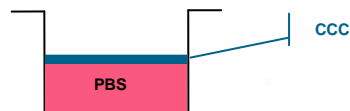
### Cell Cultivation

1. Transfer cells into a culture vessel and seed the cells in a density of 2.000 to 5.000 cells per cm<sup>2</sup>
2. Cells will attach in 2 to 4 hours
3. They will reach approximately 70 % of confluence within 4 to 6 days  
At this point they should be passaged and sub-cultured
4. Detach cells with 0.25 % Trypsin/EDTA
5. After cell detachment stop trypsinization immediately with Growth Medium
6. Count the cells
7. Seed cells with the recommended seeding density of 2.000 to 5.000 cell /cm<sup>2</sup>

## Prepare the Collagen Cell Carrier

	Ø 34mm (6-well)	Ø 14mm (24-well)	Ø 10mm(48-well)
Volume of PBS	1000µL	250µL	150µL

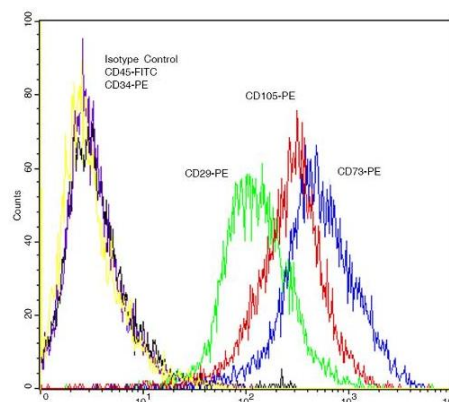
1. Preload each well with the given volume of PBS (pH 7.3 without Ca<sup>2+</sup> / Mg<sup>2+</sup>)  
Do not exceed these volumes.
2. Take a CCC with a pair of sterilized forceps out of the blister and place it onto the liquid. Do not submerge.



3. Incubate for 15 to 30 minutes at room temperature.
4. Remove the remaining PBS.  
Assure that the CCC is flatly positioned on the bottom of the well.
5. Let the plate stand in the operating laminar flow hood overnight.  
The dry CCC is slightly opaque and will become transparent again after wetting.

## Cultivation of MSC on the CCC

1. Equilibrate the dry CCC with Growth Medium (10 minutes)
2. Remove Growth Medium
3. Seed hMSC at a density of 5.000 to 10.000 cells /cm<sup>2</sup> or as desired



*hMSC analysis by flow cytometry*