

**Conditionally Immortalized Human Bone Marrow-Derived Mesenchymal Stem Cells**
**ORDER INFORMATION**

**Name of Products:** Conditionally Immortalized Human Bone Marrow-Derived Mesenchymal Stem Cells  
(**CI-HBMMSCs**)

**Catalogue Number:** **cAP-0060**

**Product Format:** Frozen Vials

**Cell Number:**  $> 5 \times 10^5/\text{vial}$

**Viability:**  $\geq 70\%$  when thawed from cryopreservation.

**General Information**

HBMMSCs (**cAP-0050**) are isolated from human Bone Marrow tissues and demonstrated with spindle-shaped-, and fibroblast-like cells. CI- HBMMSCs (**cAP-0060**) are selected from Puromycin resistant HBMMSCs after infected with lentiviruses expressing SV40 LT under the control of TetON system. CI-HGMMSCs are maintained with proliferative capacity in the presence of Doxycycline<sup>NOTE1</sup> in Mesenchymal Stem Cell Growth Medium (MSCGM, cAP-35).

**Characterization of the cells**

**Positive for** CD29, CD44, CD73, CD90, CD105, and CD166 (greater than 95% of the cell population expresses these markers by flow cytometry).

**Negative for** CD14, CD31, CD34, and CD45 (less than 2% of cell population expresses these markers by flow cytometry).

**CI-HBMMSCs** are negative for HIV-1, -2, HBV, HCV, and Bacteria, Yeast and Mycoplasma.

**Product Use:** **CI-HBMMSCs** are for Research Use Only<sup>NOTE3</sup>.

**Shipping:** Cells in Frozen Vials with Dry Ice Package.

**Handling of Arriving Cells**

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80°C freezer for short-period storage or a liquid nitrogen tank for long-term storage. Thaw the cells in a 37°C water bath, and then quickly transfer the cells into a T25 human fibronectin (10-20ug, cAP-42) flask with 10 ml MSCGM in the presence of 1ug/ml of Doxycycline and incubated overnight in a 37°C<sup>NOTE2</sup> CO2 incubator and change the medium next day (5 ml complete MSCGM) and every other thereafter.

**Subculture Protocol:**

- Rinse the cells in T25 flask with 15ml HBSS (**Room Temperature, RT**) twice.
- Add 2ml of Trypsin/EDTA (**RT**) (cAP-23) into one T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the excessive Trypsin/EDTA solution **within 30 seconds** with aspiration.
- Leave the T25 flask with the cells at **RT** for 1 minute (the cells usually will detach from the surface within 1-2 minutes). You can monitor the cells under microscope and when most of cells become rounded up, hit the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.
- Add 5ml Trypsin Neutralization Buffer and spin the cells down with 800RPM for 5 minutes.
- Re-suspend the cell pellet with 10-15 ml of MSCGM in the presence of 1ug/ml Doxycycline and the cell suspension is transferred directly into 2 or 3 fibronectin pre-coated T25 flasks (5ml each, and the cells are sub-cultured at 1:2 or 1:3 ratios)
- Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:3 ratio).

**NOTE 1:** To minimize the effect of SV40 LT antigen for your studies, cells could be cultured in the absence Doxycycline for 3-5 days before your final experiments.

**NOTE 2:** Although wild type SV40 LT antigen is used for cell immortalization, we noticed that the immortalized cells are growing better at 33-34°C, we encourage the end users to switch to 33-34°C if cells are growing slower at 37°C.

**NOTE 3:** The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

**Related products**

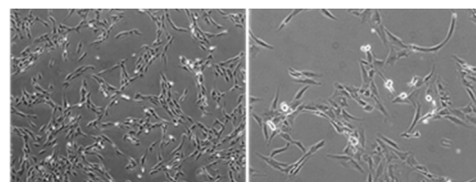
Human Serum Fibronectin	cAP-42	1.0mg	Angio-Proteomie
MSC Grown Medium (MSCGM)	cAP-35	500ml	Angio-Proteomie
MSC Basal Medium (MSCBM)	cAP-36	500ml	Angio-Proteomie
HBSS w/o $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$	cAP-11	100ml	Angio-Proteomie
Trypsin/EDTA Solution	cAP-23	100ml	Angio-Proteomie
Trypsin Neutralization Solution	cAP-28	100ml	Angio-Proteomie

**Human Material Precaution:** All tissues used for isolation are obtained under informed consent and conform to HIPAA standards to protect the privacy of the donor's personal health information. It is best to use caution when handling any human cells. We recommend that all human cells be accorded the same level of biosafety consideration as cells known to carry HIV. With infectious virus assays or viral antigen assays, even a negative test result may leave open the possible existence of a latent viral genome.

**Biosafety Level: 2**

**Angio-Proteomie Warranty:** The viability of Angio-Proteomie' cell products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. Angio-Proteomie lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the Angio-Proteomie recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the Angio-Proteomie warranty for viability is no longer valid.

**Caution:** Handling human tissue derived products is potentially bio-hazardous. Although each cell strain is tested negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate; therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.



**cAP-0060 Conditionally Immortalized Human Bone Marrow Derived Mesenchymal Stem Cells with Potent Mitogen.**