

## **GFP-Expressing Human Bone Marrow-Derived Mesenchymal Stem Cells**

### **ORDER INFORMATION**

**Name of Products:** GFP Expressing Human Bone Marrow-Derived Mesenchymal Stem Cells (**GFP-HBMMSCs**)  
**Catalogue Number:** **cAP-0050GFP**  
**Product Format:** Frozen Vials  
**Cell Number:**  $> 5 \times 10^5$ /vial  
**Viability:**  $\geq 70\%$  when thawed from cryopreservation.

### **General Information**

GFP-HBMMSCs (**cAP-0050GFP**) are selected from puromycin resistant HBMMSCs after transfected with GFP expressing lentiviral particles. GFP-HBMMSCs are supplied as either proliferative cells in a T25 flask or a cryovial at passage 3- 4. Each lot of cAP-0050GFP is tested to ensure the cells can be passaged at least for three times (i.e., approximately 9 to 10 population doublings) after thaw. GFP-HBMMSCs should be maintained in complete growth media (Mesenchymal Stem Cell Growth Medium (MSCGM), cAP-35) following the detailed protocol described below.

### **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).

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

**Product Use:** **GFP-HBMMSCs** are for research use only.

**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  $-80^{\circ}\text{C}$  freezer for short-period storage or a liquid nitrogen tank for long-term storage. Thaw the cells in a  $37^{\circ}\text{C}$  water bath, and then quickly transfer the cells into a T75 flask with 15 ml MSCGM and incubated overnight in a  $37^{\circ}\text{C}$   $\text{CO}_2$  incubator and change the medium next day (15 ml complete MSCGM) and every other thereafter.

**Subculture Protocol:**

GFP-HBMMSCs are contact inhibited. It is essential that the cells be subcultured BEFORE reaching confluence as post-confluent cells exhibit changes in morphology, slower proliferation, and reduced differentiation capacity after passaging.

- A) Rinse the cells in T75 flask with 15ml HBSS (**Room Temperature, RT**) twice.
- B) Add 4ml of Trypsin/EDTA (**RT**) (cAP-23) into one T75 flask (make sure the whole surface of the T75 flask is covered with Trypsin/EDTA), and gently dispose the excessive Trypsin/EDTA solution **within 30 seconds** with aspiration.
- C) Leave the T75 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.
- D) Add 10ml Trypsin Neutralization Buffer and spin the cells down with 800g for 5 minutes.
- E) Re-suspend the cell pellet with 30 – 45 ml of MSCGM and the cell suspension is transferred directly into 2 or 4 pre-coated T75 flasks (15ml each, and the cells are sub-cultured at 1:2 or 1:3 ratios)
- F) Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:3 ratio).

**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: 1****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.

**Related products**

Quick Coating Solution	cAP-01	240ml	Angio-Proteomie
MSC Grown Medium (MSCGM)	cAP-35	500ml	Angio-Proteomie
MSC Basal Medium (MSCBM)	cAP-36	500ml	Angio-Proteomie
HBSS w/o Ca <sup>2+</sup> , Mg <sup>2+</sup>	cAP-11	100ml	Angio-Proteomie
Trypsin/EDTA Solution	cAP-23	100ml	Angio-Proteomie
Trypsin Neutralization Solution	cAP-28	100ml	Angio-Proteomie

**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.**