

## **Canine Bone Marrow Mesenchymal Stem Cells (DBMMSCs)**

Catalogue number: **D-6043**

Suggested Medium: Mesenchymal Cell Medium /w Kit – 500 ML

Catalog No. **M5566**

### **Product Description**

Canine Bone Marrow Mesenchymal Stem Cells are derived from the tibias of beagle dog. Cells are grown in tissue culture plates with *Cell Biologics*' Culture Medium for 7-15 days. Cultures are then expanded. Prior to shipping, cells at passage 1 are detached and cryo-preserved in vials. Each vial contains  $1 \times 10^6$  cells per ml and is delivered frozen. Cells are negative for bacteria, yeast, fungi, and mycoplasma. Cells can be expanded for 2 or 3 passages at a split ratio of 1:2 under the cell culture conditions specified by *Cell Biologics*. Repeated freezing and thawing of cells is not recommended.

Canine Bone Marrow Mesenchymal Stem Cells are tested for expression of markers using antibodies, CD44, Sca-1 and CD29 by flow cytometry.

### **Laboratory Applications**

Canine Bone Marrow Mesenchymal Stem Cells can be used in standard biochemical procedures include PCR, Western blotting, immunoprecipitation, or cell derivatives for desired research applications.

### **Storage of *Cell Biologics* Products**

*Cell Biologics* ships frozen cells on dry ice. On receipt, immediately transfer frozen cells to liquid nitrogen ( $-180\text{ }^{\circ}\text{C}$ ) until ready for experimental use. Live-cell shipment is also available on request. Primary cells can never be kept at  $-20\text{ }^{\circ}\text{C}$ .

### **Authorized Uses of *Cell Biologics* Products**

Canine Bone Marrow Mesenchymal Stem Cells from *Cell Biologics* are distributed for internal research purposes only. Our products are not authorized for human use, for in vitro diagnostic procedures, or for therapeutic procedures. Transfer or resale of any *Cell Biologics*' cells or products from the purchaser to other markets, organizations or individuals is prohibited by *Cell Biologics*, without the company's written consent. *Cell Biologics*' Terms and Conditions must be accepted before submitting an order.

### **Disclaimer**

Investigators should handle the cells that they receive from *Cell Biologics* with caution and treat all Cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents.

### **Warranty and Liability**

*Cell Biologics*' guarantee applies only to your purchase of *Cell Biologics*' cells with *Cell Biologics*' Media and Coating Solution for appropriate cell culture and cell testing following *Cell Biologics*' online protocols within 35 days from the date of product delivery.

## Primary Cell Culture Protocol

All cell culture procedures must be conducted in a bio-safety cabinet.

Any and all media, supplements, and reagents must be sterilized by filtration through a 0.2 µm filter.

Use aseptic technique to prevent microbial contamination.

Cryo-preserved cells must be stored in liquid nitrogen or seeded immediately upon arrival.

### Medium

Review the information provided on the *Cell Biologics* website about appropriate culture media (e.g. serum and other supplements). Use pre-warmed (37°C) cell culture media (**30-50 ML**) to recover cryo-preserved cells and when changing media or splitting cells.

### Handling of Arriving Live Cells

When you receive the live cells in a T25 or T75 flask, remove the sticker from the filter cap, and keep the flask with 6-20 ml existing medium in 37°C CO<sub>2</sub> incubator for 1 hour before replacing the desired *Cell Biologics'* cell culture medium. Either split the 95-100% confluent cells from a T25 flask to a T75 flask after 1 hour or let the cells grow in the T25 flask with the desired Medium (such as M5566) for 12-48 hours before subculturing cells. The recommended split ratio for primary cells is 1:2.

### Cell Recovery from Cryovial

- Quickly thaw cells in cryo-vial by incubating them in a 37°C water bath for <1 min until there is just a small bit of ice left in the vial.
- Promptly remove the vial and wipe it down with 70% ethanol.
- Transfer cells from the vial to a sterile centrifuge tube. Add 8-10 ml of pre-warmed *Cell Biologics'* Cell Culture Medium.
- Flush the vial with an additional 0.5-1 ml of medium to ensure complete transfer of cells to the centrifuge tube.
- Centrifuge cells at 120 g for 5 minutes.
- Aspirate the supernatant and resuspend the cell pellet in 6 ml of *Cell Biologics'* Cell Culture Growth Medium.
- Add resuspended cells into a flask or plate.
- Place the T25 flask in a humidified, 5% CO<sub>2</sub> incubator at 37°C.
- Change culture media the following day to remove non-adherent cells and replenish nutrients.
- Change cell culture medium every day when cells are >70% confluent.
- Cells should be checked daily under a microscope to verify appropriate cell morphology.

### Expansion of Cultured Primary Cells

- Remove and discard the cell culture media from the flask.
- Flush the adherent layer 1 time using a 5 ml sterile pipette with sterile PBS (1X) without calcium and magnesium to dislodge loosely attached cells and remove fraction.
- Remove and discard the wash solution from the flask.
- Incubate cells with warm (37°C) 0.25% Trypsin-EDTA solution (*Cell Biologics*, Catalog No. 6914) for 2-5 minutes. Use 3.0 ml of Trypsin-EDTA solution when collecting cells in a T75 flask, and 2 ml when using a T25 flask. As soon as cells have detached (the flask may require a few firm gentle taps), add 8-10 ml of *Cell Biologics'* Cell Culture Medium supplemented with 5-10 % FBS to a T25 or T75 flask (the FBS will neutralize the trypsin).
- Plate cells in fresh flasks or plates precoated with Gelatin-Based Coating Solution in a humidified, 5%-CO<sub>2</sub> incubator at 37°C.

- Change culture media the following day to remove non-adherent cells and replenish nutrients.
- Cells should be checked daily under a microscopy to verify appropriate cell morphology.
- Change culture medium every 24-48 hours. Please note that the medium should be changed every day when cells are >70% confluent to remove non-adherent cells and replenish nutrients. Pre-wash cells with 1X PBS 1-2 times whenever replacing the medium.

**We recommend splitting primary cells at the follow ratio**

- The recommended split ratio for primary cells is 1:2.
- A confluent primary cells grown in a T75 flask may be expanded on a 6-well plate ready for use in experiments under the cell culture conditions specified by *Cell Biologics*.

**Procedure for Freezing Cells**

Materials:

- 1X Phosphate Buffered Saline (PBS-1X)
- 0.25% Trypsin-EDTA (1X) solution (*Cell Biologics*, Catalog No. 6914)
- Tissue Culture Media
- Cold Freezing Media (10% DMSO, 50% FBS and 40% culture medium, *Cell Biologics*, Catalog No. 6916).
- Labeled Cryovials
- Confluent Cells

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- Remove and discard the cell culture media from the flask.
  - Flush the adherent layer with a 5 ml sterile pipette 1-2 times with sterile PBS (1X) without calcium and magnesium to dislodge loosely attached cells and remove fraction.
  - Remove and discard the wash solution from the flask.
  - Incubate cells with warm (37°C) 0.25% Trypsin-EDTA solution (*Cell Biologics*, Catalog No. 6914) for 2-5 minutes. Use 3.0 ml of 0.05% Trypsin-EDTA solution when collecting cells in a T75 flask, and 2 ml when using a T25 flask. As soon as cells have detached (the flask may require a few firm gentle taps), add 10 ml of Cell Culture Medium supplemented with 5-10 % FBS to the flask (the FBS will neutralize the trypsin).
  - Centrifuge the cell suspension at 120 g for 5 minutes.
  - Remove supernatant with sterile Pasteur pipette.
  - Quickly re-suspend pellet by adding 1 ml freezing media per vial to be frozen.
  - Place vials in Nalgene "Mr. Frosty" freezing container containing 100% isopropyl alcohol at -70-80 °C for 24 h.
  - Transfer vials to liquid N<sub>2</sub> tank for indefinite storage.

**We recommend freezing primary cells at the follow ratio**

- A confluent primary cells grown in a T75 flask may be frozen in 2-3 cryovials.
- A confluent primary cells grown in a T25 flask may be frozen in 1-2 cryovials.

Please send us the cell images (>90% confluence) if you have any questions or problems with cultured cells.

Per request, a Certificate of Analysis will be provided for each cell lot purchased.