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Inspiration for Life Science

For research use only. Not for clinical diagnosis.

Compact Bone-derived Mesenchymal Stem Cells, Rat

【Rat femoral compact bone-derived, Catalog No. : MSCB01C】

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I. Product Overview

Mesenchymal stem cells (MSCs), a type of somatic stem cell, can be isolated from adipose tissue, bone marrow, umbilical cord, and dental pulp etc. MSCs are known for their excellent self-proliferation abilities, and possess the capacity to differentiate into a variety of cells, including not only bone, cartilage, and fat, but also liver[2, 3, 4, 5]. MSCs do not express MHC Class-II proteins[3]; possess immunomodulatory functions[6]; and accumulate at diseased sites, secreting various cytokines and growth factors[4], which promotes tissue regeneration and repair[5, 7].

Traditionally, bone marrow-derived MSCs (BMMSCs) have been used for research; however, isolation of MSCs from mouse bone marrow has been extremely challenging due to poor purity and yield, and due to contamination with hematopoietic cells, which can inhibit MSC proliferation[8].

In contrast, compact bone-derived MSCs (CBMSCs) are isolated by enzymatic processing of bone fragments from mouse compact bone, followed by culturing and isolating cells with minimal hematopoietic contamination, thus maintaining their multipotency and high proliferative capacity[8] for various research purposes.

This product consists of cells that have been subcultured (two passages) after being isolated from the compact bone of adult SD rat treated with collagenase. This product has been confirmed to differentiate into osteocytes, adipocytes, and chondrocytes (Note: Verification of surface antigen markers has not been conducted.).

II. Precautions Before Use

Please make sure to review this manual before using the product.

Product should be used under [aseptic operation]. The biosafety level is [Level 1].

Please use the dedicated medium sold separately for culturing this product.

III. Product Warranty

We guarantee against growth failure after the start of culture only if cells have been properly stored in liquid nitrogen, and cultured according to the manual using the dedicated medium and reagents.

The warranty period is [within 6 months of receiving the product].

Please note that the warranty does not apply if there have been changes to the medium or the method of use, if cells have been subcultured beyond the recommended number of passages, or re-frozen cells have been used.

IV. Product Components

Product Name	Catalog Nio	Size	Quantity	Storage	Expiration
Compact Bone-derived Mesenchymal Stem Cells, Rat	MSCB01C	1×10^6 cells/vial	1	Liquid nitrogen vapor phase	6 months

(This product is shipped with dry ice package. If the product is not to be used immediately upon receipt, please store it in liquid nitrogen.)

V. Cell origin

Derived from the femoral compact bone of adult SD rat.

VI. Dedicated Media (Serum-Containing Media) (sold separately)

Growth Medium

Product Name	Catalog No.	Size	Quantity	Storage	Expiration date
CBMSC Growth Medium	MSCB-GM	250 mL	1	−20 ºC (frozen) 4 ºC (after thawing)	Labeled on the bottle (when stored at −20 ℃) 3 months after thawing (when stored at 4 ℃)

(Medium's main ingredients: α-MEM, serum, antibiotics.)

Adipogenic Differentiation Medium

Product Name	Catalog No.	Size	Quantity	Storage	Expiration date
CBMSC Adipogenic Differentiation Medium	MSCB-ADDM	100 mL	1	−20 ℃ (frozen) 4 ℃ (after thawing)	Labeled on the bottle (when stored at −20 ℃) 3 months after thawing (when stored at 4 ℃)
CBMSC Adipogenic Maintenance Medium	MSCB-ADMM	250 mL	1		

Osteogenic Differentiation Medium

Product Name	Catalog No.	Size	Quantity	Storage	Expiration date
Osteogenesis Culture Medium	OGCMO	250 mL	1	−20 ℃ (frozen) 4 ℃ (after thawing)	Labeled on the bottle (when stored at −20 ℃) 3 months after thawing (when stored at 4 ℃)

Chondrogenic Differentiation Medium

Product Name	Catalog No.	Components	Size	Quantity	Storage	Expiration date
BMMSC/CBMSC Chondrogenic Differentiation Medium	MSC-CHB	Chondrogenic Differentiation Medium	50 mL	1	−20 ℃ (frozen) 4 ℃ (after thawing)	Labeled on the bottle (when stored at −20 ℃) 3 months after thawing (when stored at 4 ℃)
		Supplement	125 μL	1		Labeled on the bottle (when stored at −20 ℃) 1 month after thawing (when stored at 4 ℃)

VII. Related Products

Collagen Coating Solution

Product Name	Catalog No.	Size	Quantity
Collagen Coating Solution	SCO	100 mL	1

Cell Cryopreservation Medium

Product Name	Catalog No.	Size	Quantity
COS banker	COS-CFM01	120 mL	1

Adipocytes Staining Kit

Product Name	Catalog No.	Components	Size	Quantity
Lipid Assay Kit	AK09F	Oil Red O Solution	150 mL × 2	1 Set
		Solvent for Oil Red O	200 mL × 2	

Osteocytes Staining Kit

Product Name	Catalog No.	Components	Size	Quantity
Alkaline Phosphatase	AK20	Substrate-containing Buffer	50 mL × 1	1 Set
		Chromogenic Substrate	10 vials (For 5 mL)	
Calcified Nodule Staining Kit	AK21	Buffer (100 ×)	60 mL × 1	1 Set
		Staining Substrate	10 vials (For 10 mL)	

VIII. Instructions For Use

- **Remove the cryovial from the dry ice packaging and immediately place in liquid nitrogen until use.**
- **This product is confirmed [to be capable of being subcultured only once]. Subculturing more than twice may affect cell proliferation rate and differentiation efficiency. Therefore, customers should use their own discretion regarding its feasibility.**
- **We recommend using a collagen-coated culture vessel. This is particularly important when inducing differentiation into adipocytes, as the cells become very prone to detachment.**

IX. Protocols

Collagen Coating

[Requirements]

- Culture vessels (100-mm dishes recommended for cell proliferation; 24-well plates recommended for induction of adipocyte or osteocyte differentiation.)
- Collagen coating solution (Catalog No.: SCO)
- PBS(-) or HBSS(-)
- Sterile pipettes, conical tubes, and other culture equipment

1. Add enough collagen coating solution to cover the surface of the culture vessel (approximately 5 mL for a 100-mm dish, 0.3–0.5 mL/well for a 24-well plate); thereafter, immediately remove the solution. For glass-bottom vessels, let the solution stand overnight before removing it.
2. Wash twice with PBS(-) or HBSS(-), then add PBS(-) or HBSS(-) and let stand for at least one hour before use. If not using immediately, fill the vessel with PBS(-) or HBSS(-) and store at room temperature for several days.

Cell Thawing and Seeding

※ For culturing in 100-mm dishes.

[Requirements]

- Compact Bone-derived Mesenchymal Stem Cells, Rat (Catalog No.: MSCB01C)
- CBMSC Growth Medium (Catalog No.: MSCB-GM)
- Three collagen-coated 100-mm dishes
- Sterile pipettes, conical tubes, and other culture equipment

1. Remove the cryovial containing the frozen cells from liquid nitrogen storage, and immediately place it in a 37 ℃ water bath. Quickly thaw the cells (within 90–120 seconds) by gently swirling the vial in the 37 ℃ water bath until only a small bit of ice remains in the vial.
2. Transfer the vial to a clean bench. Before opening, wipe the outside of the vial with 70% ethanol.
3. Transfer the thawed cell solution to a 15-mL conical tube containing 10 mL of pre-warmed (37 ℃) growth medium. After gentle mixing, centrifuge the solution at 200 ×g for 5 minutes at room temperature.
4. Remove and discard the supernatant, then add 9 mL of pre-warmed (37 ℃) growth medium and

resuspend the cells.

5. Add 3 mL of the cell suspension and 7 mL of pre-warmed (37 ℃) growth medium each to three 100-mm dishes pre-coated with collagen. Gently mix to evenly disperse the cells. Then, culture the cells in a 37 ℃ incubator with 5% CO_2.
6. Three days after seeding of cells (the 3rd day of culture), replace the medium with pre-warmed (37 ℃) growth medium. Thereafter, replace the medium every 1–2 days with pre-warmed (37 ℃) growth medium (Fig. 1) **(Note: Do not replace the medium until 3 days after seeding to allow time for the cells to adhere to the culture vessel.)**.
7. Once 70–80% confluency is achieved (the 3rd to 7th day of culture), proceed with passaging, differentiation induction experiments, or freeze the cells for storage.

Cell Subculturing

※ For subculturing from 100-mm dishes.

[Requirements]

- Cells at 70–80% confluency
- CBMSC Growth Medium (Catalog No.: MSCB-GM)
- PBS(-) or HBSS(-)
- 0.25% trypsin-EDTA solution
- Collagen-coated culture vessels
- Sterile pipettes, conical tubes, and other culture equipment

1. Remove and discard the remaining growth medium from the culture vessel containing cells at 70–80% confluency (the 3rd to 7th day of culture), wash the vessel with pre-warmed (37 ℃) PBS(-) or HBSS(-) 1–2 times.
2. Add 3 mL of 0.25% trypsin-EDTA solution and incubate the cells at 37 ℃ for 2–3 minutes while observing.
3. Gently tap the culture vessel horizontally, then check for cell detachment under a microscope **(Note: Extending trypsin-EDTA treatment beyond 3 minutes may affect cell condition. Cells not detached are strongly adhered to the vessel, and should not be forcibly detached to avoid damage or denaturation.)**.
4. Add 10 mL of pre-warmed (37 ℃) growth medium to inactivate trypsin, and pipette to collect cells into a conical tube.
5. Centrifuge at 200 ×g for 5 minutes at room temperature, remove and discard the supernatant, add an appropriate amount of pre-warmed (37 ℃) growth medium, and resuspend and count the cells.
6. After counting the cells, adjust to the required cell density for seeding, differentiation induction experiments, etc.

Cryopreservation of Cells

[Requirements]

- Cells at 70–80% confluency
- CBMSC Growth Medium (Catalog No.: MSCB-GM)
- PBS(-) or HBSS(-)
- 0.25% trypsin-EDTA solution
- COS banker (Cell cryopreservation medium) (Catalog No.: COS-CFM01)
- Cryovial
- Cell freezing container (Product name: CoolCell FTS30, Manufacturer: BM Equipment Co, Ltd., Catalog No.: BCS-170 etc.)
- Sterile pipettes, conical tubes, and other culture equipment

1. Perform steps 1 to 5 of "Cell Subculturing."
2. Centrifuge again at 200 ×g for 5 minutes at room temperature, remove and discard the supernatant, add pre-cooled (4 ℃) cell cryopreservation medium, and resuspend to achieve 1 × 10^6 cells/mL.
3. Dispense aliquots of the cell suspension into cryovials and freeze at −80 ℃ in a cell freezing container.
4. Transfer to liquid nitrogen storage the following day.

Adipogenic Differentiation

※ For culturing in 24-well plates.

[Requirements]

- Collagen-coated 24 well plates
- Cells at 70–80% confluency
- CBMSC Growth Medium (Catalog No.: MSCB-GM)
- CBMSC Adipogenic Differentiation Medium (Catalog No.: MSCB-ADDM)
- CBMSC Adipogenic Maintenance Medium (Catalog No.: MSCB-ADMM)
- Sterile pipettes, conical tubes, and other culture equipment
- Lipid Assay Kit (Catalog No.: AK09F)

1. Following "Cell Thawing and Seeding" or "Cell Subculturing", seed cells in a collagen-coated 24-well plate (seeding density: 1 × 10^4 cells/cm^2), and culture in growth medium (0.5 mL/well) until 70–80% confluency is achieved.
2. Replace the medium with pre-warmed (37 ℃) adipogenic differentiation medium (0.5 mL/well) and culture for 3 days.
3. Replace the medium with pre-warmed (37 ℃) adipogenic maintenance medium (0.5 mL/well) and culture for 2 days.
4. Small lipid droplets will start appearing at this stage.
5. Thereafter, replace the medium every 2–3 days with pre-warmed (37 ℃) adipogenic maintenance medium (0.5 mL/well), and observe the growth of the lipid droplets.
6. Accumulated lipid droplets will maximize in approximately 7 days. The accumulated lipid droplets can

be stained with a Lipid Assay Kit (Fig. 2).

Osteogenic Differentiation

※ For culturing in 24-well plates.

[Requirements]

- Collagen-coated 24 well plates
- Cells at 70–80% confluency
- CBMSC Growth Medium (Catalog No.: MSCB-GM)
- Osteogenesis Culture Medium (Catalog No.: OGCMO)
- Sterile pipettes, conical tubes, and other culture equipment
- Alkaline Phosphatase Staining Kit (Catalog No.: AK20)
- Calcified Nodule Staining Kit (Catalog No.: AK21)

1. Following “Cell Thawing and Seeding” or “Cell Subculturing”, seed cells in a collagen-coated 24-well plate (seeding density: 1×10^4 cells/cm^2), and culture in growth medium (0.5 mL/well) until 70–80% confluency is achieved.
2. Replace the medium with pre-warmed (37 ℃) osteogenesis culture medium (0.5 mL/well) and continue to culture.
3. Thereafter, replace the medium every 2–3 days with pre-warmed (37 ℃) osteogenesis culture medium (0.5 mL/well) and observe the growth of the osteocytes.
4. Osteogenesis will occur in 2–3 weeks. The formed osteocyte can be stained with the Alkaline Phosphatase Staining Kit (Fig. 3) or the Calcified Nodule Staining Kit (Fig. 4).

Chondrogenic Differentiation

[Requirements]

- Collagen-coated 100-mm dishes
- Cells at 70–80% confluency
- CBMSC Growth Medium (Catalog No.: MSCB-GM)
- BMMSC/CBMSC Chondrogenic Differentiation Medium (Catalog No.: MSC-CHB)
- Sterile pipettes, conical tubes, and other culture equipment

1. Before use, thaw the chondrogenic differentiation medium (50 mL) at 4 ℃, and add one bottle of thawed supplement.
2. Following "Cell Thawing and Seeding" or "Cell Subculturing", seed cells in a collagen-coated 100-mm dish, and culture in growth medium until 70–80% confluency is achieved.
3. Perform steps 1 to 5 of "Cell Subculturing".
4. Centrifuge again at 200 ×g for 5 minutes at room temperature, remove and discard the supernatant, add pre-warmed (37 ℃) chondrogenic differentiation medium, and resuspend the cells to achieve 5 × 10^5 cells/mL.
5. Dispense 0.5 mL of the resuspended cells in 15-mL conical tubes (2.5 × 10^5 cells/tube).
6. Centrifuge at 300 ×g for 5 minutes at room temperature, leave the supernatant, loosen the lid of the 15-mL conical tube slightly on a clean bench for gas exchange, and culture in a 37 ℃ incubator with 5% CO_2 **(Note: Do not resuspend the cells as they are cultured in pellet form.)**.
7. After 24 hours, replace the medium with pre-warmed (37 ℃) chondrogenic differentiation medium (0.5 mL/well).
8. Thereafter, replace the medium every 2–3 days with pre-warmed (37 ℃) chondrogenic differentiation medium (0.5 mL/well). If the pellet adheres to the side of the 15-mL conical tube, gently tap the tube to detach the pellet.
9. Chondrocyte aggregates will form in 3–4 weeks. The formed chondrocyte aggregates can be fixed with formalin, embedded in paraffin, and then stained with Alcian Blue, etc. (Fig. 5).

X. Cell Morphology

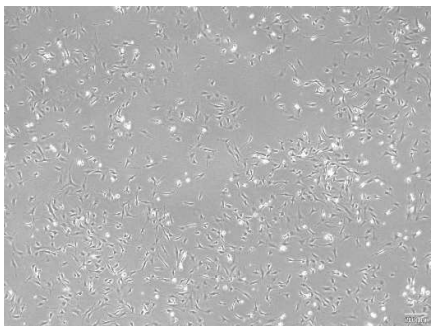

Fig. 1 Cells on the 3rd day of culture (Culturing in growth medium)

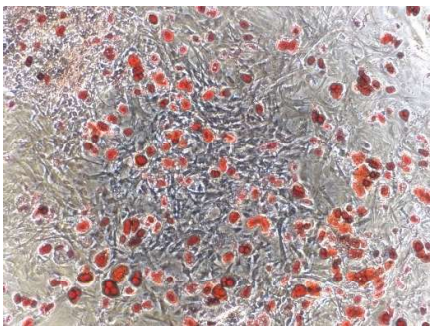

Fig. 2 Adipogenically differentiated cells (Staining with Lipid Assay Kit)

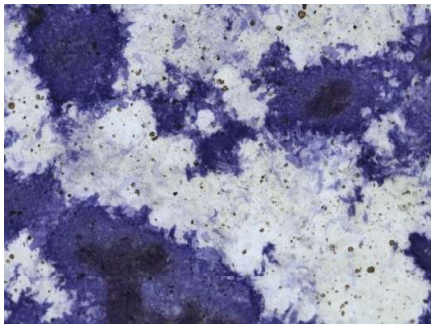

Fig. 3 Osteogenically differentiated cells (Staining with Alkaline Phosphatase Staining Kit)

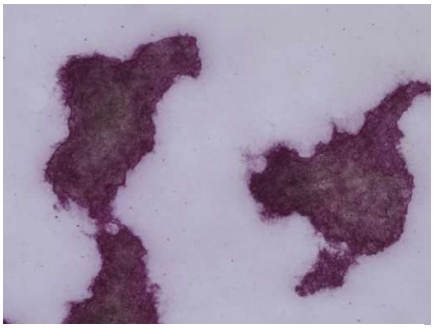

Fig. 4 Osteogenically differentiated cells (Staining with Calcified Nodule Staining Kit)

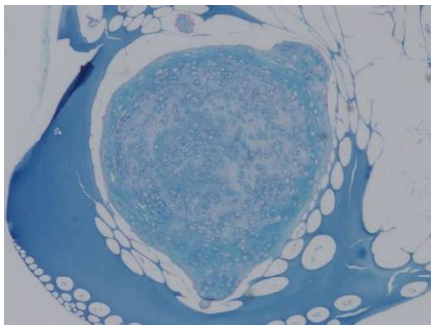

Fig. 5 Chondrogenically differentiated cells (Staining with Alcian Blue)

XI. References

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