StemPro™ BM mesenchymal stem cells

Catalog Numbers A15652 and A15652

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Description

StemPro™ BM Mesenchymal Stem Cells (MSCs) were isolated from human bone marrow (donated with informed consent) and expanded under hypoxic (3–5% O_2) conditions. Before cryopreservation at passage 4 (P4), the cells were tested for viability (>90% post-thaw viability), expression of cell surface markers indicative of MSC (i.e., CD73+, CD90+, CD105+, CD166+), and their ability to differentiate into osteocytes, adipocytes, and chondrocytes.

Product	Catalog No.	Amount	Storage
StemPro™ BM Mesenchymal Stem Cells	A15652	1×10^6 viable cells/vial	–196 to –150°C
	A15653	5×10^6 viable cells/vial	

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.

Important information

- StemPro™ BM MSCs may be cultured in StemPro™ MSC SFM XenoFree, StemPro™ MSC SFM CTS™, or MesenPRO RS™ Medium.
- Xenofree culture of StemPro™ BM MSCs requires pre-coated culture vessels for successful recovery and expansion. However, StemPro™ BM MSCs can be cultured in MesenPRO RS™ Medium without the need for pre-coated culture vessels. To culture the cells in MesenPRO RS™ Medium, follow the same procedure as described for StemPro™ MSC SFM XF below, but omit the steps for coating the culture vessels.
- The protocol below describes the culture of StemPro™ BM MSCs in StemPro™ MSC SFM XF. As for any culture system, seeding density should be optimized for each media system.

Culture conditions

Media: StemPro™ MSC SFM XenoFree medium

Culture Type: Adherent

Recommended Substrate: CELLStart™ CTS™ substrate

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5% CO_2 under hypoxic (5% O_2) or normoxic (20% O_2) conditions.

Coat culture vessels with CELLStart™ CTS™ substrate

1. Dilute the CELLStart™ CTS™ solution 1:100 in DPBS with calcium and magnesium (i.e., 100 µL substrate into 10 mL of DPBS). Pipet gently to mix. *Do not vortex*.

2. Coat each culture vessel with the appropriate volume of CELLStart™ CTS™ solution (see Table 1).

Table 1 Reagent volumes (in mL per well or per dish)

Culture vessel (approx. surface area)	CELLStart™ CTS™ reagent (1:100)	Complete medium	TrypLE™ enzyme solution
6-well (10 cm^2/well)	2 mL	2–3 mL	0.5–1 mL
12-well (4 cm^2/well)	1 mL	1–2 mL	0.2–0.5 mL
24-well (2 cm^2/well)	0.5 mL	0.5–1 mL	0.1–0.2 mL
35-mm (10 cm^2)	0.5–1 mL	2–3 mL	0.5–1 mL
60-mm (20 cm^2)	2–3 mL	3–5 mL	1–2 mL
100-mm (60 cm^2)	7–10 mL	10–15 mL	2–3 mL
T-25 (25 cm^2)	2–3 mL	4–5 mL	1–2 mL
T-75 (75 cm^2)	7–10 mL	12–15 mL	2–3 mL

3. Place the culture vessel with CELLStart™ CTS™ solution in a 37°C incubator with a humidified atmosphere of 5% CO_2 for 1 hour.

4. After incubation, remove the vessel from the incubator and temporarily place it in a laminar flow hood until use. Immediately before use, remove all CELLStart™ CTS™ solution and replace it with complete medium.

Note: Do not store diluted CELLStart™ CTS™ solution; prepare a fresh solution before every use. Pre-coated vessels can be stored at 4°C, wrapped with Parafilm™ laboratory film to avoid drying.

Prepare culture medium

StemPro™ MSC SFM Basal Medium requires supplementation with StemPro™ MSC SFM XenoFree Supplement and GlutaMAX™-I CTS™ supplement.

1. For 500 mL complete medium, aseptically add 5 mL of StemPro™ MSC SFM XenoFree Supplement to StemPro™ MSC SFM Basal Medium (500 mL).

2. Aseptically add 5 mL GlutaMAX™-I CTS™ supplement to the complete medium before use.

3. If so desired, add 50 µL of 50 mg/mL Gentamicin reagent solution to the complete medium.

Recovery

1. Rapidly thaw a frozen vial of StemPro™ BM MSCs in a 37°C water bath until a small amount of ice remains.

2. Pipet the entire contents of the vial into a 15-mL or a 50-mL conical tube.

3. Carefully add 5–10 mL of pre-warmed (37°C) complete StemPro™ MSC SFM XF medium to the conical tube at a rate of approximately 3 to 5 drops per 5 seconds and gently swirl after every addition.

4. Centrifuge the cells at 100–200 × *g* for 5 minutes at room temperature.

5. Aspirate the supernatant, resuspend the cells in a minimal volume of pre-warmed complete StemPro™ MSC SFM XF medium, and determine the viable cell density using your preferred method (e.g., Countess™ automated cell counter).

6. Remove the CELLStart™ CTS™ solution from the coated vessel and add the appropriate amount of complete StemPro™ MSC SFM XF medium (see Table 1).

7. Seed the vessel with 5×10^3 cells/cm^2 (e.g., 3.75×10^5 cells/T75 flask). Mix or swirl the cell suspension to ensure an even distribution.

8. Place the culture vessel in a 37°C incubator with a humidified atmosphere of 5% CO_2.

9. Replace the spent medium every 2 days with fresh pre-warmed complete StemPro™ MSC SFM XF medium.

Subculture

1. Observe the culture vessel under the microscope and confirm that the cells are ready to be passaged (~60–90% confluent).

2. Pre-warm TrypLE™ Select CTS™ reagent and complete StemPro™ MSC SFM XF medium to 37°C before use.

3. Remove the spent medium from culture vessel and discard.

4. Wash the cell surface with DPBS without calcium and magnesium.

5. Add the appropriate amount of TrypLE™ Select CTS™ reagent to the vessel (see Table 1) and tilt the vessel in all directions to evenly distribute the reagent. Incubate the cells for 3–7 minutes in the 37°C incubator.

6. After incubation, check the vessel under the microscope for cell detachment. Firmly tap the flask as necessary to facilitate complete cell detachment.

7. Add the same volume of pre-warmed StemPro™ MSC SFM XF medium as the TrypLE™ Select CTS™ reagent to the vessel, pipet up and down over surface to detach the cells, and transfer the cell suspension into a centrifuge tube.

8. Centrifuge tube at 100–200 × *g* for 5 minutes at room temperature.

9. Aspirate the supernatant, resuspend the cells in a minimal volume of pre-warmed complete StemPro™ MSC SFM XF medium, and determine the viable cell density using your preferred method (e.g., Countess™ automated cell counter).

10. Remove the CELLStart™ CTS™ solution from the coated vessel and add the appropriate amount of complete StemPro™ MSC SFM XF medium (see Table 1).

11. Seed the vessel with 5×10^3 cells/cm^2 (e.g., 3.75×10^5 cells/T75 flask). Mix or swirl cell suspension to ensure even distribution.

12. Place the culture vessel in a 37°C incubator with a humidified atmosphere of 5% CO_2.

13. Replace the spent medium every 2 days with fresh pre-warmed complete StemPro™ MSC SFM XF medium.

Related products

Product	Cat. No.
StemPro™ MSC SFM XenoFree	A10675
StemPro™ MSC SFM CTS™	A10332
MesenPRO RS™ Medium	12746
CELLStart™ CTS™	A10142
GlutaMAX™-I CTS™, (100X), liquid	A12860
Gentamicin (50 mg/mL)	15750
TrypLE™ Select CTS™ (1X), liquid	A12859
DPBS CTS™ with calcium, magnesium (1X), liquid	A12858
DPBS CTS™ without calcium, magnesium (1X), liquid	A12856
Countess™ Automated Cell Counter	C10227
StemPro™ Adipogenesis Differentiation Kit	A10070-01
StemPro™ Osteogenesis Differentiation Kit	A10072-01
StemPro™ Chondrogenesis Differentiation Kit	A10071-01

Reference

Vertelov, G., Kharazi, L., Muralidhar, M.G., Sanati, G., Tankovich, T., and A. Kharazi. 2013. High targeted migration of human mesenchymal stem cells grown in hypoxia is associated with enhanced activation of RhoA. *Stem Cell Res Ther*. 4:5.

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