Culture of MSCs on Nunclon™ Supra Surface Cultureware

Catalog Numbers 150476, 140680, 167013, 156376, 156380, 140682, 150478, 140681, 156372, 150470, 150472, 150474, 140683, 156374, 156378, 156382

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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.

Product description

The Thermo Scientific™ Nunclon™ Supra Surface treatment is used for culturing a variety of fastidious, adherent cells and supports serum-free and coating-free cultures of human mesenchymal stromal cells (MSCs). MSCs cultured on the Nunclon™ Supra Surface retain their morphology and downstream differentiation potential. Nunclon™ Supra Surface cultureware used in conjunction with Gibco™ StemPro™ MSC SFM complete medium is a xeno-free solution for cell therapy research applications.

Contents and storage

Product	Cat. No.	Storage	Shelf Life[1]
Nunclon™ Supra Surface Cultureware	150476, 140680, 167013, 156376, 156380, 140682, 150478, 140681, 156372, 150470, 150472, 150474, 140683, 156374, 156378, 156382	Ambient	5 years

[1] Shelf life is determined from the date of manufacture.

Product use

- Nunclon™ Supra Surface cultureware: For laboratory use.
- StemPro™ MSC SFM XenoFree: For Research Use Only. Not for use in diagnostic procedures.

Important information

CAUTION! Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HBsAg. Handle in accordance with established bio-safety practices.

Procedural guidelines

- Thaw StemPro™ MSC SFM XenoFree Supplement overnight at 2°C to 8°C prior to use (thawed supplement will have a slightly cloudy appearance). Use thawed material immediately or aliquot (i.e., 1 mL) unused material and store at –20°C to –5°C. Avoid additional freeze-thaw cycles.
- StemPro™ MSC SFM XenoFree complete medium (StemPro™ MSC SFM Basal Medium, StemPro™ MSC SFM XenoFree Supplement, and CTS™ GlutaMAX™ Supplement) is stable for 2 weeks when stored in the dark at 2°C to 8°C.

Prepare complete medium

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

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 CTS™ GlutaMAX™ Supplement or 200 mM L-Glutamine to the complete medium before use.
3. *Optional*: add 50 µL of 50 mg/mL Gentamicin to the complete medium.

Culture conditions

Culture vessels: Nunclon™ Supra Surface cultureware

Media: StemPro™ MSC SFM XenoFree complete medium

Cell line(s): Human mesenchymal stem cells (MSCs) or adipose-derived stem cells (ADSCs)

Culture type: Adherent

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 4–6% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Note: Procedures detailed in the following sections are for cultures in T-75 culture flasks (75 cm^2). Volumes should be adjusted accordingly for the desired vessel size.

Recover cryopreserved human MSCs

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

2. Pipet the entire contents of the cryovial into a 50-mL conical tube.

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

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

5. Resuspend the cell pellet in pre-warmed (37°C) StemPro™ MSC SFM XenoFree complete medium and add the cell suspension to an appropriate Nunclon™ Supra Surface flask at a density of ≥ 5 × 10^3 cells/cm^2

   Note: For the initial isolation of MSCs, supplementation of the complete medium with 2.5% Human Serum, Type AB facilitates cell attachment and growth. For subsequent passages, Human Serum, Type AB is not required

6. Incubate at 36°C to 38°C in a humidified atmosphere of 4–6% CO_2 in air.

7. Replace the medium in the flasks every 2 days.

Guidelines for subculturing cells in StemPro™ MSC SFM XenoFree complete medium

- StemPro™ MSC SFM XenoFree has been developed for the multi-passage expansion of human bone marrow-derived MSCs and adipose-derived stem cells (ADSCs) at greater than clonal densities (≥ 5 × 10^3 cells/cm^2). Reduced seeding densities may result in suboptimal cell expansion, although optimal growth conditions must be determined for each application.
- When subculturing human MSCs in StemPro™ MSC SFM XenoFree, input cell confluence should be 60–90%, cell viability should be at least 90% and the growth rate should be in mid-logarithmic phase prior to subculture. Initiating cultures under suboptimal conditions may affect product performance. Transitioning MSCs or ADSCs from serum-containing medium to StemPro™ MSC SFM XenoFree does not require an adaptation protocol.
- For optimal performance, re-feed the cultures every 2 days with StemPro™ MSC SFM XenoFree complete medium

Note: Procedures detailed on the next section are for cultures maintained in a Nunclon™ Supra Surface T-75 culture flask (75 cm^2). Adjust the volumes accordingly for desired vessel size.

Propagate cells on Nunclon™ Supra Surface cultureware

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

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

3. Add 10 mL of pre-warmed StemPro™ MSC SFM XenoFree complete medium to a 50-mL conical tube for each flask being harvested.

4. Remove the spent medium from the T-75 flask and discard.

5. Wash the cell surface with 10 mL of CTS™ DPBS without calcium chloride, without magnesium chloride, remove and discard.

6. Add 3–5 mL of CTS™ TrypLE™ Select Enzyme to the T-75 flask and tilt the flask in all directions to evenly distribute. Incubate the cells in CTS™ TrypLE™ Select Enzyme for 2–10 minutes in the incubator.

   Note: Cells coming out of serum-containing medium may require a longer incubation time (5–10 minutes), while cells growing under serum-free conditions should detach more readily (2–3 minutes).

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

8. Add 7 mL of pre-warmed StemPro™ MSC SFM XenoFree to each flask and collect the cell suspension in the 50-mL conical tube containing complete medium from step 3. Firmly tap the flask, re-wash with 10 mL StemPro™ MSC SFM XenoFree and collect.

   Note: The addition of StemPro™ MSC SFM XenoFree complete medium to harvested cells is critical for preventing the cells from adhering to the wall of the conical tube during centrifugation.

9. Centrifuge the tubes at 100–200 × *g* for 5 minutes at room temperature.

10. Resuspend the cells in a minimal volume of pre-warmed StemPro™ MSC SFM XenoFree complete medium for cell counting, using a preferred counting method (e.g., Countess™ 3 FL Automated Cell Counter).

11. Add 15 mL of StemPro™ MSC SFM XenoFree complete medium to as many new Nunclon™ Supra Surface flasks as desired.

12. Add enough cell suspension to each flask to provide ≥ 5 × 10^3 cells/cm^2 (i.e. 3.75 × 10^5 cells per T-75 flask). Mix or swirl the cell suspension to ensure even distribution.

13. Place the culture flask(s) in the incubator at 36°C to 38°C with a humidified atmosphere of 4–6% CO_2.

14. Replace the spent culture medium every 2 days with pre-warmed StemPro™ MSC SFM XenoFree complete medium.

Cryopreservation

1. Prepare cryopreservation solution (2X) by supplementing StemPro™ MSC SFM XenoFree complete medium with 10–20% Dimethyl Sulfoxide (DMSO). Store at 4°C until use; make cryopreservation medium on day of intended use.

2. Reconstitute thre harvested cell pellet to twice the desired final concentration (i.e., 2 × 10^6 cells/mL) in pre-warmed StemPro™ MSC SFM XenoFree complete medium.

3. Slowly add cryopreservation solution to the cell suspension, and gently mix to ensure even cell distribution.

4. Immediately add the desired volume of cell suspension (i.e., 1 mL) to pre-chilled (2°C to 8°C) cryovials.

5. Place the cryovials at -70°C in a cryogenic freezing container (e.g., "Mr. Frosty™ Freezing Container (1°C)).

6. After 24 hours, transfer the frozen cells to liquid nitrogen (vapor phase); storage at -200°C to -125°C is recommended.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Product	Catalog No.
StemPro™ MSC SFM XenoFree	A1067501
CTS™ GlutaMAX™ Supplement	A1286001
L-Glutamine (200 mM)	A2916801
Gentamicin	15710064
CTS™ TrypLE™ Select Enzyme	A1285901
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
Human Serum, Type AB, Fisher BioReagents	BP2525100
Thermo Scientific™ Nunc™ EasYFlask™ Flasks with Nunclon™ Supra Surface: • Filter cap: T-25, T-75, T-175 • Solid cap; T-25, T-75, T-175	156372, 156376, 156380, 156374, 156378, 156382
Thermo Scientific™ Nunc™ Multidishes with Nunclon™ Supra Surface 12-well, 6-well, 24-well, 48-well	140681, 140680, 140682, 140683
Thermo Scientific™ Nunc™ EasYDish™ Dishes with Nunclon™ Supra Surface, 35-mm, 60-mm, 100-mm, 100-mm, 150-mm	150470, 150472, 150474, 150476, 150478
Countess™ 3 FL Automated Cell Counter	AMQAF2001

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. A MAN1000717

Revision	Date	Description
A	21 November 2024	Initial release.