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StemPro™ Human Adipose-Derived Stem Cells

Catalog Numbers R7788110 and R7788115

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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**.

Introduction

StemPro™ Human Adipose-Derived Stem Cells (ADSCs) are isolated from human adipose tissue collected during liposuction procedures and cryopreserved from primary cultures. Before cryopreservation, the ADSCs are expanded for one passage in MesenPRO RS™ Medium. Each lot of ADSCs originates from a single donor of human lipoaspirate tissue.

Each vial of ADSCs contains cells that can differentiate into multiple mature cell phenotypes *in vitro*, including adipocytes, osteoblasts, and chondrocytes. *In vitro* differentiation into non-mesenchymal cell types, such as neuronal and glial progenitors, hepatocytes and vascular endothelial progenitors have also been described. In addition, ADSCs are known to secrete pro-angiogenic, immunomodulatory and anti-apoptotic factors.

MesenPRO RS™ Medium is recommended for use with these cells for optimal growth and expansion.

Table 1 StemPro™ Human Adipose-Derived Stem Cell Kit (R7788110)

Kit Components	Amount	Storage
StemPro™ Human Adipose-Derived Stem Cells (1 × 10^6 cells/mL)	1 mL	Liquid nitrogen, vapor-phase
MesenPRO RS™ Basal Medium	500 mL	Store at 2℃ to 8℃; Protect from light
MesenPRO RS™ Growth Supplement	10 mL	Store at -5℃ to -20℃; Protect from light

Table 2 StemPro™ Human Adipose-Derived Stem Cells (R7788115)

Kit Components	Amount	Storage
StemPro™ Human Adipose-Derived Stem Cells (1 × 10^6 cells/mL)	1 mL	Liquid nitrogen, vapor-phase

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**.

General cell handling

CAUTION! As with other human cell lines, when working with ADSCs, handle as potentially biohazardous material under at least Biosafety Level 1 containment.

IMPORTANT! It is very important to strictly follow the guidelines for culturing ADSCs in this manual to keep them undifferentiated.

- All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.
- Before starting experiments, ensure cells have been established (at least 1 passage), and also have some frozen stocks on hand.
- For differentiation studies and other experiments, we recommend using cells below passage 5.

- For general maintenance of cells, cell confluency should be 60–80%, cell viability should be at least 90%, and the growth rate should be in mid-logarithmic phase prior to subculturing.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.
- Antibiotic-antimycotic containing penicillin, streptomycin, and amphotericin B may be used if required.

Prepare complete MesenPRO RS™ medium

Thaw MesenPRO RS™ Growth Supplement at 2 to 8°C prior to use. Avoid repeated freeze-thaw cycles of the supplement

1. Aseptically add 10 ml of MesenPRO RS™ Growth Supplement to 500 ml of MesenPRO RS™ Basal Medium
2. Aseptically add L-glutamine to the medium to a final concentration of 2 mM (*e.g.*, add 5 ml of 200 mM L-glutamine stock to 500 ml of medium).

Do not store the prepared complete MesenPRO RS™ Medium longer than 15 days.

Thaw the StemPro™ Adipose-Derived Stem Cells

1. Prewarm prepared Complete MesenPRO RS™ Medium to 37℃.
2. Remove the cells from liquid nitrogen storage, and wipe the cryovial with ethanol or isopropanol before opening. In an aseptic field, briefly twist the cap a quarter turn to relieve pressure and then retighten. Do not expose cells to air before thawing.
3. Quickly thaw the vial of cells by swirling it in a 37℃ water bath. Remove the cells immediately when the last bit of ice has melted, typically < 2 minutes. Do not submerge the vial completely. Do not thaw the cells for longer than 2 minutes.
4. When thawed, immediately transfer cells into a 15-ml sterile conical tube and dropwise add 1 ml of prewarmed Complete MesenPRO RS™ Medium with gentle mixing.
5. Plate the cells (2 ml) on a tissue-culture treated 35-mm dish. The recommended seeding density for Adipose-Derived Stem Cells is 5,000 cells per cm^2.
6. Incubate at 37℃, 5% CO_2 and 90% humidity and allow cells to adhere for several hours (or overnight).
7. When the cells have attached to the growth surface, replace the medium with an equal volume of fresh, prewarmed Complete MesenPRO RS™ Medium.
8. Change the medium every 3–4 days.

Passage the StemPro™ Adipose-Derived Stem Cells

1. Aspirate the Complete MesenPRO RS™ Medium from the cells.
2. Rinse the surface of the cell layer with DPBS (approximately 2 ml DPBS per 10 cm^2 culture surface area), by adding the DPBS to the side of the vessel opposite the attached cell layer and rocking back and forth several times.
3. Remove the DPBS by aspiration and discard.
4. To detach the cells, add a sufficient volume of prewarmed TrypLE™ Express without phenol red to cover the cell layer (approximately 0.5 ml per 10 cm^2).
5. Incubate at 37˚C for approximately 7 minutes.
6. Observe the cells under a microscope. If the cells are less than 90% detached, continue incubating and observe within 2 minutes for complete detachment of the cells. Tap the vessel to expedite cell detachment.
7. When ≥ 90% of the cells have detached, tilt the vessel for a minimal length of time to allow the cells to drain. Add the equivalent of 2 volumes (twice the volume used for the TrypLE™ Express) of temperature-equilibrated Complete MesenPRO RS™ Medium.
8. Disperse the medium by pipetting over the cell layer surface several times.

9. Transfer the cells to a 15-ml conical tube and centrifuge at 210 × *g* for 5 minutes at room temperature.

10. Resuspend the cell pellet in a minimal volume of temperature-equilibrated Complete MesenPRO RS™ Medium and remove a sample for counting.

11. Determine the total number of cells and percent viability using a hemacytometer, cell counter and Trypan Blue exclusion, or the Countess™ automated cell counter. If necessary, add Complete MesenPRO RS™ Medium to the cells to achieve the desired cell concentration and recount the cells.

12. Determine the total number of vessels to inoculate by using the following equation:

    Number of vessels = Number of viable cells ÷ (growth area of vessel in cm^2 × 5,000 cells per cm^2 recommended seeding density)

13. Add Complete MesenPRO RS™ Medium to each vessel so that the final culture volume is 0.2–0.5 ml per cm^2.

14. Add the appropriate volume of cells to each vessel and incubate at 37℃, 5% CO_2 and 90% humidity.

15. Three to four days after seeding, completely remove the medium. Replace with an equal volume of Complete MesenPRO RS™ Medium.

Freeze the StemPro™ Adipose-Derived Stem Cells

- Freeze cells at a density of 1×10^6–2×10^6 viable cells/ml.
- Use a freezing medium composed of final concentrations of 20% Fetal Bovine Serum (MSC Cell-qualified) and 10% DMSO.
- Bring the cells into freezing medium in two steps, as described in this section.

Prepare the freezing media

Prepare Freezing Medium A and B immediately before use. You will need enough of each freezing medium to resuspend cells at a density of 1×10^6–2×10^6 cells/ml.

1. In a sterile 15-ml tube, mix together the following reagents for every 1 ml of **Freezing Medium A** needed:

Complete MesenPRO RS™ Medium	0.6 ml
Fetal Bovine Serum, MSC-Qualified	0.4 ml

2. In another sterile 15-ml tube, mix together the following reagents for every 1 ml of **Freezing Medium B** needed:

Complete MesenPRO RS™ Medium	0.8 ml
DMSO	0.2 ml

3. Place tube with Freezing Medium B on ice until use (leave Freezing Medium A at Room Temperature).

   Note: Discard any remaining freezing medium after use.

Freeze the cells

1. Aspirate Complete MesenPRO RS™ Medium from the flask, well, or dish.

2. Rinse the surface with DPBS (approximately 2 ml DPBS per 10 cm^2 culture surface area) by adding the DPBS to the side of the vessel opposite the attached cell layer and rocking back and forth several times.

3. Remove the DPBS by aspiration and discard.

4. To detach the cells, add a sufficient volume of prewarmed TrypLE™ Express without phenol red to cover the cell layer (approximately 0.5 ml per 10 cm^2).

5. Incubate at 37℃ for approximately 7 minutes.

6. Observe the cells under a microscope. If the cells are less than 90% detached, continue incubating and observe within 2 minutes for complete detachment of the cells. Tap the vessel to expedite cell detachment.

7. When ≥90% of the cells have detached, tilt the vessels on end for a minimal length of time to allow the cells to drain. Add the equivalent of 2 volumes (twice the volume used for the TrypLE™ Express) of temperature-equilibrated Complete MesenPRO RS™ Medium to each vessel.

8. Disperse the medium by pipetting over the cell layer surface several times.

9. Transfer the cells to a 15-ml conical tube and centrifuge at 210 × *g* for 5 minutes at room temperature.

10. Resuspend the cell pellet in a minimal volume of temperature-equilibrated Complete MesenPRO RS™ Medium and remove a sample for counting.

11. Determine the total number of cells electronically using the Countess™ automated cell counter or a Coulter Counter, or manually using a hemacytometer and an inverted microscope.

12. Gently aspirate media from the vessel and resuspend the cells to a concentration of 4×10^6 cells/ml in Freezing Medium A.

13. Add the same volume of Freezing Medium B to cells in a **dropwise** manner.

14. Aliquot 1 ml to each freezing vial and store at –80℃ overnight in an isopropanol chamber.

15. The next day, transfer the frozen vials to a liquid nitrogen tank for long-term storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen.

Osteogenic differentiation

This section provides media-preparation guidelines and a protocol for inducing StemPro™ ADSCs to differentiate towards osteoblasts using the StemPro™ Osteogenesis Differentiation Kit (Cat. No. A1007201).

Prepare the complete differentiation medium

To prepare Complete StemPro™ Osteogenesis Differentiation Medium, thaw the StemPro™ Osteogenesis Supplement at 4℃, room temperature, or in a 37℃ water bath, and prepare as below.

Store complete medium at 2–8℃ in the dark.

Component	Final Conc.	For 100 ml
StemPro™ Osteocyte/Chondrocyte Differentiation Basal Medium	1X	90 ml
StemPro™ Osteogenesis Supplement	1X	10 ml
Gentamicin (10 mg/ml)	5 µg/ml	50 µl

Prepare an osteogenic cell culture

1. Observe the ADSC monolayer to ensure mid-log growth phase confluence (60–80%). Aspirate the medium and floating cells from the culture flask and discard.

2. Add 5–10 ml DPBS to the flask. Gently rinse the cell monolayer.

3. Remove DPBS and add 5–7 ml of pre-warmed TrypLE™ Express to the flask and completely coat the culture surface. Incubate for 5–8 minutes at 36–38℃ or until cells have fully detached.

4. Gently pipet detached cells into a single-cell solution and verify on inverted microscope.

5. Remove the cell suspension from the flask, transfer into a centrifuge tube, and pellet cells at 100 × *g* for 5–10 minutes.

6. Determine cell viability and total cell density electronically using the Countess™ automated cell counter or a Coulter Counter, or manually using a hemacytometer and an inverted microscope.

7. Resuspend the pellet in an appropriate volume of pre-warmed Complete MesenPRO RS™ Medium.

8. Seed the ADSCs into culture vessels at 5×10^3 cells/cm^2. For classical stain differentiation assays, seed into a 12-well plate. For gene-expression profile studies, seed into a T-75 flask. For immunocytochemistry studies, seed into a 16-well CultureWell™ chambered coverglass or 96-well plate.

9. Incubate in Complete MesenPRO RS™ Medium at 36–38℃ in a humidified atmosphere of 4–6% CO_2 for a minimum of 2 hours up to 4 days.

10. Replace media with pre-warmed Complete StemPro™ Osteogenesis Differentiation Medium and continue incubation. ADSCs will continue to expand as they differentiate under osteogenic conditions. Refeed cultures every 3–4 days.

11. After specific periods of cultivation, osteogenic cultures can be processed for alkaline phosphatase staining (7–14 days) or Alizarin Red S staining (>21 days), gene expression analysis, or protein detection.

Adipogenic differentiation

This section provides media-preparation guidelines and a protocol for inducing StemPro™ ADSCs to differentiate towards adipocytes using the StemPro™ Adipogenesis Differentiation Kit (Cat. No. A1007001).

Prepare the adipogenic differentiation medium

To prepare the complete medium, thaw the supplement in a 37±2℃ water bath, swirl and warm the supplement to promote dissolution of the precipitate, and prepare the medium as described in the table below. Store complete medium at 2–8℃ in the dark.

Component	Final Conc.	For 100 ml
StemPro™ Adipocyte Differentiation Basal Medium	1X	90 ml
StemPro™ Adipogenesis Supplement	1X	10 ml
Gentamicin (10 mg/ml)	5 µg/ml	50 µl

Note: It is normal to see a precipitate formed in the supplement after thawing. To promote dissolution of the precipitate, warm the supplement with swirling for no more than 30 minutes prior to preparing complete media. Any remaining precipitate should be suspended in solution before it is added to StemPro™ Adipocyte Differentiation Basal Medium, and will dissolve completely when mixed with the Basal Medium and warmed.

Prepare an adipogenic cell culture

1. Observe the ADSC monolayer to ensure mid-log growth phase confluence (60–80%). Aspirate the medium and floating cells from culture flask and discard.

2. Add 5–10 ml DPBS. Gently rinse the cell monolayer.

3. Remove the DPBS and add 5–7 ml of pre-warmed TrypLE™ Express to the flask and completely coat the culture surface. Incubate for 5–8 minutes at 36–38℃ or until cells have fully detached.

4. Gently pipet the detached cells into a single-cell solution and verify on inverted microscope.

5. Remove the cell suspension from the flask, transfer into a centrifuge tube, and pellet cells at $100 \times g$ for 5–10 minutes.

6. Determine cell viability and total cell density electronically using the Countess™ automated cell counter or a Coulter Counter, or manually using a hemacytometer and an inverted microscope.

7. Resuspend the pellet in an appropriate volume of pre-warmed Complete MesenPRO RS™ Medium.

8. Seed the ADSCs into culture vessels at 1×10^4 cells/cm^2. For classical stain differentiation assay, seed into a 12 well plate. For gene expression profile studies, seed into a T-75 flask. For immunocytochemistry studies, seed into a 16-well CultureWell™ chambered coverglass or 96-well plate.

9. Incubate in Complete MesenPRO RS™ Medium at 36–38℃ in a humidified atmosphere of 4–6% CO_2 for a minimum of 2 hours up to 4 days.

10. Replace media with pre-warmed Complete Adipogenesis Differentiation Medium and continue incubation. ADSCs will continue to undergo limited expansion as they differentiate under adipogenic conditions. Refeed cultures every 3–4 days.

11. After specific periods of cultivation, adipogenic cultures can be processed for Oil Red O or LipidTOX™ staining (beginning at 7–14 days), gene expression analysis or protein detection.

Chondrogenic differentiation media and methods

This section provides media-preparation guidelines and a protocol for inducing StemPro™ ADSCs to differentiate towards chondrocytes using the StemPro™ Chondrogenesis Differentiation Kit (Cat. No. A1007101).

Prepare the chondrogenesis differentiation medium

To prepare Complete StemPro™ Chodrogenesis Differentiation Medium, thaw the StemPro™ Chondrogenesis Supplement at 4℃, room temperature, or in a 37℃ water bath, and prepare as below.

Store complete medium at 2–8℃ in the dark.

Component	Final Conc.	For 100 ml
StemPro™ Osteocyte/Chondrocyte Differentiation Basal Medium	1X	90 ml
StemPro™ Chondrogenesis Supplement	1X	10 ml
Gentamicin (10 mg/ml)	5 µg/ml	50 µl

Prepare a chondrogenic cell culture

1. Observe cell monolayer from basal cultures expanded in StemPro™ MSC SFM™, MesenPRO RS™ medium, or standard growth medium (DMEM + 10% FBS) to ensure mid-log growth-phase confluence (60 to 80%). Aspirate medium and floating cells from culture flask and discard.

2. Add 5 to 10 mL DPBS. Gently rinse cell monolayer.

3. Remove DPBS, add 5 to 7 mL of pre-warmed TrypLE™ Express to flask, and completely coat the culture surface. Incubate for 5 to 8 minutes at 36 to 38℃ or until cells have fully detached.

4. Gently pipet detached cells into a single cell solution and verify on inverted microscope.

5. Remove cell suspension from flask, transfer into a centrifuge tube, and pellet cells at 100 × *g* for 5 to 10 minutes.

6. Determine cell viability and total cell density electronically using the Countess™ automated cell counter or a Coulter Counter, or manually using a hemacytometer and an inverted microscope.

7. For MesenPRO RS™ expansion cultures, resuspend pellet in an appropriate volume of pre-warmed MesenPRO RS™ media to generate a cell solution of 1.6×10^7 viable cells/ml. For StemPro™ MSC SFM™ or standard growth medium, use MSC Attachment Medium (see above) to generate a cell solution of 1.6×10^7 viable cells/ml.

8. Generate micromass cultures by seeding 5-µl droplets of cell solution in the center of multi-well plate wells for classical stain or 100-mm Petri dish for gene expression analysis, protein detection, or immunohistochemistry.

9. After cultivating micromass cultures for 2 hours under high humidity conditions, add warmed chondrogenesis media to culture vessels and incubate in 37℃ incubator with 5% CO_2.

10. Refeed cultures every 2 to 3 days.

11. After specific periods of cultivation, chondrogenic pellets can be processed for Alcian Blue or Safranin O staining (>14 days), gene expression analysis, protein detection, or immunohistochemistry.

Troubleshooting

Problem	Cause	Solution
No viable cells after thawing stock	Stock not stored correctly	Order new stock and store in liquid nitrogen. Keep in liquid nitrogen until thawing.
	Home-made stock not viable	Freeze cells at a density of 1×10^6–2×10^6 viable cells/ml.
		Use low-passage cells to make your own stocks.
		Slow freezing and fast thawing is the key. Add Freezing Medium B drop wise manner (slowly). At time of thawing, thaw quickly and do not expose vial to the air but quickly change from nitrogen tank to 37℃ water bath.
		Obtain new StemPro™ ADSCs.
	Thawing medium not correct	Use prewarmed Complete MesenPRO RS™ Medium.
	Cells too diluted	Generally we recommend thawing one vial in a 35-mm dish at a density of 5,000 cells per cm^2.
Cells grow slowly	Growth medium not correct	Use prewarmed Complete MesenPRO RS™ Medium.
	Cells too old	Use healthy ADSCs, under passage 5; do not overgrow.
Cells differentiated	Culture conditions not correct	Thaw and culture fresh vial of StemPro™ ADSCs. Follow thawing instructions and subculture procedures exactly.
	Cells too old	ADSCs above passage 5 may become differentiated.

Additional products

Item	Cat. no.
MesenPRO RS™ Medium (includes Basal Medium and Growth Supplement)	12746-012
GlutaMAX™-1 Supplement	35050-061
StemPro™ MSC SFM™	A10332-01
StemPro™ Osteogenesis Differentiation Kit	A1007201
StemPro™ Chondrogenesis Differentiation Kit	A1007101
StemPro™ Adipogenesis Differentiation Kit	A1007001
Gentamicin (10 mg/ml)	15710-064
Dulbecco's Phosphate Buffered Saline (DPBS), containing no calcium, magnesium, or phenol red	14190-144
Fetal Bovine Serum, MSC-Qualified	12662-011
TrypLE™ Express without phenol red	12604-013
Antibiotic-Antimycotic (100X), liquid	15240-062
Dulbecco's Modified Eagle Medium (DMEM) (1X) (low glucose) with 1,000 mg/l D-glucose and 110 mg/l sodium pyruvate—without L-glutamine and phenol red	11054-020
Dulbecco's Modified Eagle Medium (DMEM) (1X) (high glucose) with 4.5 g/l D-glucose and sodium pyruvate— without L-glutamine	10313-021
L-glutamine (200 mM, liquid)	25030-081
Countess™ Automated Cell Counter	C10227
CD 31 Mouse Anti-Human, Purified	MHCD3100
CD 90 Purified MS X HU	AHU0051
CD 29, Mouse Anti-Human, Purified	CD2900
CD 14 Mouse Anti-Human, Purified	MHCD1400
CD 105 Mouse Anti-Human, Purified	MHCD10500
CD 44 Mouse Anti-Human, Purified	MHCD4400
CD 45 Mouse Anti-Human, Purified	MHCD4500
CD 73 (Host: Mouse, Clone: 7G2)	41-0200

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