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by life technologies

StemPro® Adipogenesis Differentiation Kit

Description

The StemPro® Adipogenesis Differentiation Kit has been developed for the adipogenic differentiation of mesenchymal stem cells (MSCs) in tissue culture vessels. The kit contains all reagents required for inducing MSCs to be committed to the adipogenesis pathway and generate adipocytes. Using StemPro® Adipogenesis Differentiation Kit in combination with StemPro® MSC SFM or MesenPRO RS™ Medium provides a standardized culture workflow solution for MSC isolation, expansion, and differentiation into matrix-forming adipocytes.

Product	Catalog no.	Amount	Storage	Shelf life*
StemPro® Adipogenesis Differentiation Kit Contains:	A10070-01	1 kit	—	—
StemPro® Adipogenesis Differentiation Basal Medium	A10410-01	100 mL	2°C to 8°C; Protect from light	12 months
StemPro® Adipogenesis Supplement	A10065-01	10 mL	–20°C to –5°C; In the dark	12 months

* Shelf life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

- StemPro® Adipogenesis Supplement is supplied frozen. Thaw prior to use, as described in **Prepare media**.
- It is normal to see a precipitate formed in the supplement after thawing. The precipitate does not impact performance of the product. See **Prepare media** for guidelines for dissolving the precipitate.
- Thawed StemPro® Adipogenesis Supplement is stable up to at least one month at 2°C to 8°C. *Do not refreeze the supplement after thawing.*
- Complete StemPro® Adipogenesis Differentiation Medium is stable up to at least one month at 2°C to 8°C.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare media

Complete Adipogenesis Differentiation Medium

Thaw the supplement in a 37±2°C water bath, and prepare 100 mL of media according to the following table. Store complete medium at 2°C to 8°C in the dark.

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. Suspend any remaining precipitate in solution before adding it to StemPro® Adipocyte Differentiation Basal Medium; remaining precipitate will dissolve completely when mixed with the Basal Medium and warmed.

Adipogenesis Differentiation Medium	Concentration	Volume
StemPro® Adipocyte Differentiation Basal Medium	1X	90 mL
StemPro® Adipocyte Supplement	1X	10 mL
Gentamicin reagent (10 mg/mL)	5 µg/mL	50 µL

MSC growth medium

Prepare MSC growth medium according to the following table.

MSC growth medium (500 mL)	Final concentration	Volume
DMEM low glucose	—	445 mL
MSC-qualified FBS	10%	50 mL
GlutaMAX™-I (200 mM)	2 mM	5 mL
Gentamicin reagent (10 mg/mL)	5 µg/mL	250 µL

Culture conditions

Media: StemPro® Adipogenesis Differentiation Medium.
Cell line: Human mesenchymal stem cells.
Culture type: Adherent
Culture vessels: 12-well tissue-culture plates, 16-well CultureWell™ slides, 96-well tissue-culture plates, 75 cm^2 tissue culture flasks
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.

Important guidelines for adipogenesis differentiation

- Expansion culture: Expand primary MSC isolates with StemPro® MSC SFM or MesenPRO RS™ Medium in T-75 or T-150 flasks. We have successfully used standard growth media of DMEM+10% MSC Qualified FBS. We recommend refeeding the cultures every 2–3 days and passaging every 5–7 days.
- Passaging: We strongly recommend using low-passage MSCs (<8 to 10 passages). Continuously passaged MSCs will gradually lose their multipotency with increased passage number (>10 passages).
- Harvesting: We recommend using TrypLE™ Express for enzymatically treating and harvesting MSCs. TrypLE™ Express is a recombinant protease that has been demonstrated to be gentle on MSCs. Overexposure to trypsin will lead to reduced MSC viability and expansion.
- Timing of passaging: Do not let passaged MSCs become completely confluent, as it can reduce multipotency of MSCs. Passage cultures when they reach 60–80% confluency, cell viability is at least 90%, and the growth rate is in mid-logarithmic phase.
- Seeding density: For expansion, we recommend a seeding density of 3×10^3 to 5×10^3 viable cells/cm^2 with MesenPRO RS™ Medium or 1×10^4 viable cells/cm^2 with StemPro® MSC SFM.
- Confluency: Expanding MSCs in growth medium for 2–4 days before refeeding with Complete Adipogenesis Differentiation Medium can enhance adipogenesis.

Adipogenesis differentiation

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 (60–80%). Aspirate medium and floating cells from culture flask and discard.
2. Add 5–10 mL DPBS. Gently rinse cell monolayer.
3. Remove DPBS, add 5–7 mL of pre-warmed TrypLE™ Express to flask and completely coat culture surface. Incubate for 5–8 minutes at 36°C to 38°C 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 5–10 minutes.

6. Determine cell viability and total cell density using Trypan Blue Stain and an electronic (Coulter Counter) or manual (hemocytometer) cell counting method.
7. Resuspend the pellet in an appropriate volume of pre-warmed MSC Growth Medium (see **Prepare media**, page 1).
8. Seed MSCs 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 the cells in MSC Growth Medium at 36°C to 38°C 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. MSCs 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; see the following section for method), gene expression analysis, or protein detection.

HCS LipidTOX™ Green neutral lipid stain analysis

1. After 7 days or longer under differentiating conditions, remove the media from the 16-well CultureWell™ or 96-well tissue culture plate and rinse once with DPBS. Fix cells with 4% formaldehyde solution for 30 minutes.
2. After fixation, rinse wells twice with DPBS, apply 1:100 dilution LipidTOX™ Green and incubate for 15–30 minutes. You can add 1:4000 Hoechst 33342 as a nuclear counterstain.
3. Rinse twice with DPBS, apply SlowFade® Gold to the wells, visualize under fluorescent microscope and capture images for qualitative or quantitative analysis.

Images of cells in StemPro® Adipogenesis Differentiation Medium

Figure 1 Analysis of MSCs cultured in StemPro® Adipogenesis Differentiation Medium demonstrated differentiation into adipogenic lineage by Oil Red O and HCS LipidTOX™ Green neutral lipid staining.

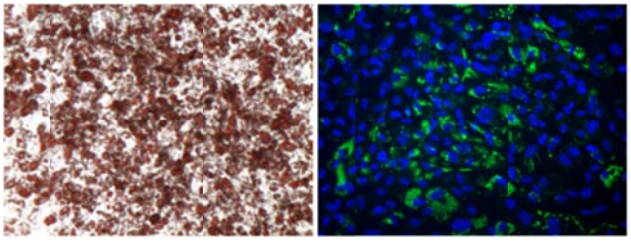
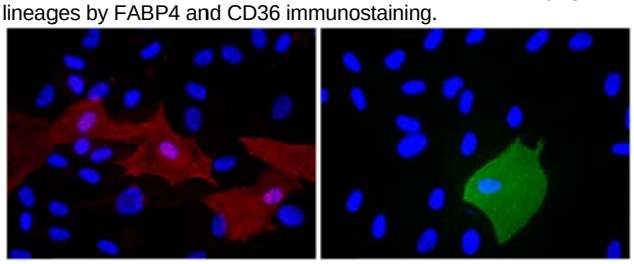


Figure 2 Analysis of MSCs cultured in StemPro® Adipogenesis Differentiation Medium demonstrated differentiation into adipogenic lineages by FABP4 and CD36 immunostaining.



Related products

Product	Catalog no.
CTS™ StemPRO® MSC SFM	A10332
StemPRO® Human Adipose-Derived Stem Cell Kit	R7788
StemPRO® Osteogenesis Differentiation Kit	A10072-01
MesenPRO RS™ Medium	12746
FBS, MSC-Qualified (non-US)	12662
GlutaMAX™-I	35050
Gentamicin reagent (10 mg/mL)	15710
TrypLE™ Express	12604
DPBS without Ca^{++} and Mg^{++}	14190
HCS LipidTOX™ Green neutral lipid	H34475
SlowFade® Gold antifade reagent	S2828
CultureWell™ chambered coverglass	C37005
Trypan Blue Solution, 0.4%	15250

Explanation of symbols and warnings

The symbols present on the product label are explained below:

			
Temperature Limitation	Batch code	Use By:	Catalog number
			
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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