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STEMPRO® Adipogenesis Differentiation Kit

Introduction

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 lipid vesicle-forming adipocytes.

Description	Cat. no.	Size	Storage	Shelf Life
STEMPRO® Adipogenesis Differentiation Kit	A10070-01	1 kit		—
Contains:				
STEMPRO® Adipocyte Differentiation Basal Medium	A10410-01	100 mL	2 to 8°C (protect from light)	12 months
STEMPRO® Adipogenesis Supplement	A10065-01	10 mL	–5 to –20°C (in the dark)	12 months

Intended Use

For research use only (RUO). **Caution:** Not intended for human or animal diagnostic or therapeutic uses.

Characteristics

The STEMPRO® Adipogenesis Differentiation Kit has been extensively tested and proven to have the following characteristics:

- Contains all components required to reliably and reproducibly induce MSCs into the adipogenic lineage.
- Demonstrated to robustly induce adipogenesis in adipose tissue-derived stem cells (STEMPRO® Human Adipose-Derived Stem Cell Kit, Cat. nos. R7788-110 and R7788-115).
- Lipid vesicle staining methods demonstrates differentiation of MSCs into adipocytes (Figure 1).
- Immunocytochemistry methods demonstrate expression of relevant adipocyte biomarkers (Figure 2).

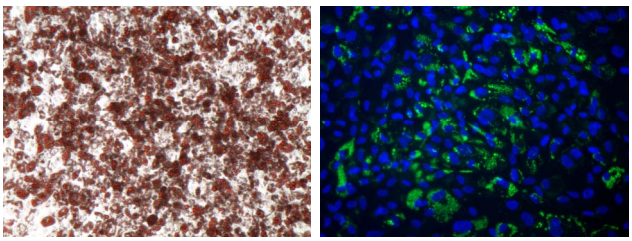

Figure 1: Analysis of MSCs cultured in STEMPRO® Adipogenesis Differentiation Medium demonstrated differentiation into adipogenic lineage by Oil Red O and HCS LipidTOX™ Green neutral lipid staining.

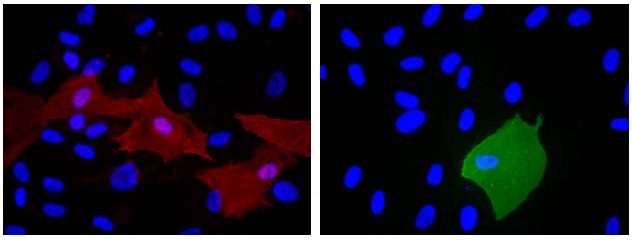

Figure 2: Analysis of MSCs cultured in STEMPRO® Adipogenesis Differentiation Medium demonstrated differentiation into adipogenic lineages by FABP4 and CD36 immunostaining.

Storage and Handling

- STEMPRO® Adipogenesis Supplement is supplied frozen. Thaw supplement in a 37±2°C water bath prior to use.
- **It is normal to see a precipitate formed in the supplement after thawing.** The precipitate does not impact performance of the product. See **Media Preparation**, next page, for guidelines for dissolving the precipitate.
- **Do not refreeze the supplement after thawing.** Thawed STEMPRO® Adipogenesis Supplement is stable up to at least one month at 2 to 8°C.
- Complete STEMPRO® Adipogenesis Differentiation Media is stable up to at least one month at 2 to 8°C.

Important Guidelines for Adipogenesis Differentiation

To obtain optimal adipogenic differentiation with STEMPRO® Adipogenesis Differentiation Medium, follow these guidelines:

- **Expansion culture:** Primary MSC isolates should be expanded with STEMPRO® MSC SFM or MesenPRO RS™ Medium in T-75 or T-150 flasks. Standard growth media of DMEM+10% MSC Qualified FBS has been successfully tested. It is recommended that the cultures are refed every 2 to 3 days and passaged every 5 to 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**: It is critical to not let passaged MSCs become completely confluent as it can reduce multipotency of MSCs. Passaging should take place when cultures reach 60 to 80% confluency, cell viability be at least 90% and growth rate be 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 to 4 days (to near or complete confluency) before refeeding with Adipogenesis Differentiation Medium can enhance adipogenesis.

Certificate of Analysis

The Certificate of Analysis (CofA) provides quality control information for this product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on the box.

Physical Conditions for Adipogenesis Culture

Media: STEMPRO® Adipogenesis Differentiation Medium
Cell Line: Human mesenchymal stem cells
Incubator: 36 to 38°C, humidified atmosphere of 4 to 6% CO_2 in air
Culture Conditions: Adherent; ensure proper gas exchange and minimize exposure to light
Recommended Culture Vessels: 12-well tissue-culture plates, 16-well CultureWell slides, 96-well tissue-culture plates, 75 cm^2 tissue-culture flasks

Media Preparation

Complete Adipogenesis Differentiation Medium: Prepare according to the table below. Thaw supplement in 37±2°C water bath. Store complete medium at 2 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. 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.

Adipogenesis Differentiation Medium	Conc.	100 mL
STEMPRO® Adipocyte Differentiation Basal Medium	1X	90 mL
STEMPRO® Adipogenesis Supplement	1X	10 mL
Gentamicin reagent (10 mg/mL)	5 µg/ml	50 µl

MSC Growth Medium: Prepare as below.

MSC Growth Medium	Final Conc.	For 500 mL
DMEM low glucose		445 mL
MSC-qualified FBS	10%	50 mL
GLUTAMAX™-I (200 mM)	2 mM	5 mL
Gentamicin (10 mg/mL)	5 µg/mL	250 µl

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 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 culture surface. Incubate for 5 to 8 minutes at 36 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 to 10 minutes.
6. Determine cell viability and total cell density using Trypan Blue Stain and electronic (*i.e.,* Coulter Counter) or manual (*i.e.,* hemocytometer) cell counting method.
7. Resuspend pellet in appropriate volume of pre-warmed MSC Growth Medium (see **Media Preparation**).
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 in MSC Growth Medium at 36 to 38°C in a humidified atmosphere of 4 to 6 % CO_2 for a minimum of 2 hours up to 4 days.
10. Replace media with pre-warmed Adipogenesis Differentiation Medium and continue incubation. MSCs will continue to undergo limited expansion as they differentiate under adipogenic conditions. Refeed cultures every 3 to 4 days.
11. After specific periods of cultivation, adipogenic cultures can be processed for Oil Red O or LipidTOX™ staining (beginning at 7 to 14 days; see below for method), gene expression analysis or protein detection.

HCS LipidTOX™ Green Neutral Lipid Stain Analysis

1. After 7 days or longer under differentiating condition, remove media from 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 to 30 minutes. 1:4000 Hoechst 33342 can be added 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.

Additional Products

Some products are recommended but not supplied in the kit. See below for ordering information.

Product	Size	Cat. no.
STEMPRO® MSC SFM	500 mL	A10332-01
STEMPRO® Human Adipose-Derived Stem Cell Kit	1 kit	R7788
STEMPRO® Osteogenesis Differentiation Kit	1 kit	A10072-01
MesenPRO RS™ Medium	500 mL	12746
FBS, MSC-Qualified (non-US)	100 mL	12662
GLUTAMAX™-I	100 mL	35050
Gentamicin reagent (10 mg/mL)	10 mL	15710
TrypLE™ Express	100 mL	12604
DPBS without Ca^{++} and Mg^{++}	500 mL	14190
HCS LipidTOX™ Green neutral lipid	1 kit	H34475
SlowFade® Gold antifade reagent	1 kit	S-2828
CultureWell™ chambered coverglass	1 pack	C-37005
Trypan Blue Stain	100 mL	15250

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

Purchaser Notification

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