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STEMPRO® Chondrogenesis Differentiation Kit

Introduction

The STEMPRO® Chondrogenesis Differentiation Kit has been developed for the chondrogenic differentiation of mesenchymal stem cells (MSCs) in tissue-culture vessels. The kit contains all reagents required for inducing MSCs to be committed to the chondrogenesis pathway and generate chondrocytes. Using STEMPRO® Chondrogenesis 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 collagen matrix-producing chondrocytes.

Description	Cat. no.	Size	Storage	Shelf Life
STEMPRO® Chondrogenesis Differentiation Kit	A10071-01	1 kit		—
Contains:				
STEMPRO® Osteocyte/Chondrocyte Differentiation Basal Medium	A10069-01	100 mL	2 to 8°C (protect from light)	12 months
STEMPRO® Chondrogenesis Supplement	A10064-01	10 mL	–5 to –20°C (in the dark)	12 months

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.

Intended Use

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

Characteristics

The STEMPRO® Chondrogenesis 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 chondrogenic lineage.
- Demonstrated to robustly induce chondrogenesis in adipose tissue-derived stem cells (STEMPRO® Human Adipose-Derived Stem Cell Kit, Cat. no. R7788-110 and R7788-115).
- Classical staining methods demonstrate differentiation of MSCs into chondrocytes (Figure 1).

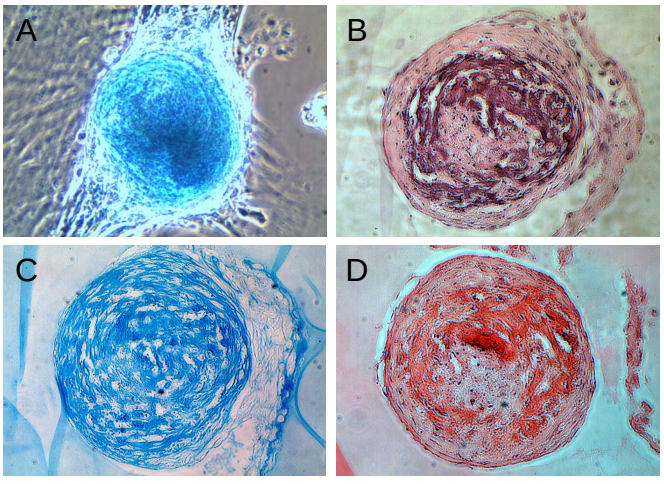

Figure 1: Analysis of MSCs cultured in STEMPRO® Chondrogenesis Differentiation Medium demonstrated differentiation into chondrogenic lineage by A) Alcian Blue staining of developing chondrogenic pellet, B) hematoxylin staining of cross-section of day 20 chondrogenic pellet, C) Alcian Blue staining of cross-section of same day 20 chondrogenic pellet, and D) Safranin O staining of cross-section of same day 20 chondrogenic pellet.

Storage and Handling

- STEMPRO® Chondrogenesis Supplement is supplied frozen. Thaw prior to use, as described in **Media Preparation**, next page.
- Thawed STEMPRO® Chondrogenesis Supplement is stable up to at least one month at 2 to 8°C. Supplement can be refrozen in desired volumes and stored at –5°C to –20°C. **Avoid multiple freeze thaw cycles** of supplement.
- Store prepared Complete STEMPRO® Chondrogenesis Differentiation Medium at 2 to 8°C in the dark. Complete medium is stable up to at least one month at 2 to 8°C.

Important Guidelines for Chondrogenesis Differentiation

To obtain optimal chondrogenic differentiation with STEMPRO® Chondrogenesis 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-225 flasks. Standard growth media of DMEM+10% MSC Qualified FBS has been successfully tested. We recommend refeeding the cultures every 2 to 3 days and passaging 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. Passage cultures when they reach 60 to 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.
- **Micromass culture:** We recommend preparing a cell solution of 1.6×10^7 viable cells/ml and letting cells attach to culture surface under humidified conditions for 2 hours before adding Chondrogenesis Differentiation Media.

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/support, and is searchable by product lot number, which is printed on the box label.

Physical Conditions for Chondrogenesis Culture

Media: STEMPRO® Chondrogenesis 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, 24-well tissue-culture plates, or 100-cm^2 tissue-culture plates

Media Preparation

Complete Chondrogenesis Differentiation Medium: Thaw supplement at 4°C, room temperature, or in a 37°C water bath, and prepare as below. Store complete medium at 2 to 8°C in the dark.

Chondrogenesis Differentiation Medium	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

MSC Attachment Medium: Prepare as below.

MSC Attachment Medium	Final Conc.	For 100 mL
DMEM low glucose		89 mL
MSC-qualified FBS	10%	10 mL
GLUTAMAX™-I (200 mM)	2 mM	1 mL
Gentamicin (10 mg/mL)	5 µg/mL	50 µl

Chondrogenesis 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 the 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. 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 x 10^7 viable cells/ml. For STEMPRO® MSC SFM or standard growth medium, use MSC Attachment Medium (see **Media Preparation**) to generate a cell solution of 1.6 x 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°C 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.

Alcian Blue Stain Analysis

1. After 14 days or longer under differentiating conditions, remove media from culture vessel, rinse once with DPBS, and fix cells with 4% formaldehyde solution for 30 minutes.
2. After fixation, rinse wells with DPBS and stain cells with 1% Alcian Blue solution prepared in 0.1 N HCL for 30 minutes.
3. Rinse wells three times with 0.1 N HCl, add distilled water to neutralize the acidity, visualize under light microscope, and capture images for analysis. Blue staining indicates synthesis of proteoglycans by chondrocytes.

Additional Products

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

Product	Size	Cat. no.
STEMPRO® MSC SFM	1 kit	A10332-01
STEMPRO® Human Adipose-Derived Stem Cell Kit	1 kit	R7788
MesenPRO RS™ Kit	1 kit	12746-012
DMEM low glucose	500 mL	11054
FBS, MSC-Qualified (non-US)	100 mL	12662
GLUTAMAX™-I	100 mL	35050
CELLstart™	2 ml	A10142-01
Gentamicin (10 mg/mL)	10 mL	15710
TrypLE™ Express	100 mL	12604
DPBS without Ca^{++} and Mg^{++}	500 mL	14190
Collagenase Type II	1 g	17101
Trypan Blue Stain	100 mL	15250
Mouse anti-Aggrecan	0.5 mg	AHP0012
Mouse anti-Aggrecan	0.5 mL	AHP0022
Mouse anti-CD29	100 µg	AHS2902
Mouse anti-Osteonectin/SPARC	100 µg	33-5500
STEMPRO® Adipogenesis Differentiation Kit	1 kit	A10070-01
STEMPRO® Osteogenesis Differentiation Kit	1 kit	A10072-01

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

Purchaser Notification

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