gibco
by life technologies

StemPro® Chondrogenesis Differentiation Kit

Description

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 the 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 matrix-forming chondrocytes.

Product	Catalog no.	Amount	Storage	Shelf life*
StemPro® Chondrogenesis Differentiation Kit Contains:	A10071-01	1 kit	—	—
StemPro® Osteocyte/Chondrocyte Differentiation Basal Medium	A10069-01	100 mL	2°C to 8°C; Protect from light	12 months
StemPro® Chondrogenesis Supplement	A10064-01	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® Chondrogenesis Supplement is supplied frozen. Thaw prior to use, as described in **Prepare media**.
- Thawed StemPro® Chondrogenesis Supplement is stable up to at least one month at 2°C to 8°C. You can refreeze the supplement in aliquots; store aliquots at –20°C to –5°C. *Avoid multiple freeze thaw cycles of supplement.*
- Store prepared Complete StemPro® Chondrogenesis Differentiation Medium at 2°C to 8°C in the dark. Complete 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.

StemPro® Chondrogenesis Differentiation Supplement only

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

Prepare media

Complete Chondrogenesis Differentiation Medium

Thaw the supplement at 4°C, room temperature, or in a 37°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.

Chondrogenesis Differentiation Medium	Concentration	Volume
StemPro® Osteocyte/Chondrocyte Differentiation Basal Medium	1X	90 mL
StemPro® Chondrogenesis 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 (100 mL)	Final concentration	Volume
DMEM low glucose	—	89 mL
MSC-qualified FBS	10%	10 mL
GlutaMAX™-I (200 mM)	2 mM	1mL
Gentamicin reagent (10 mg/mL)	5 µg/mL	50 µL

Culture conditions

Media: StemPro® Chondrogenesis Differentiation Medium

Cell line: Human mesenchymal stem cells

Culture type: Adherent

Culture vessels: 12-well tissue-culture plates, 24-well tissue-culture plates, or 100-cm^2 tissue-culture plates

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 Chondrogenesis Differentiation

- Expansion culture: Expand primary MSC isolates with StemPro® MSC SFM or MesenPRO RS™ Medium in T-75 or T-225 flasks. We have successfully tested 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.
- Micromass culture: We recommend preparing a cell solution of 1.6×10^7 viable cells/mL and letting cells attach to the culture surface under humidified conditions for 2 hours before adding Chondrogenesis Differentiation Media.

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 (60–80%). 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 $100 \times g$ 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. For MesenPRO™ RS expansion cultures, resuspend the 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 **Prepare media**, page 1) 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.

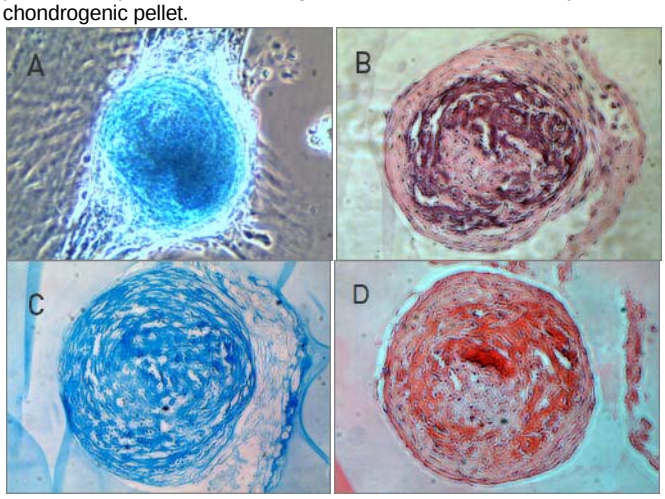
9. After cultivating micromass cultures for 2 hours under high humidity conditions, add warmed chondrogenesis media to culture vessels and incubate in 37°C incubator with 5% CO_2.
10. Refeed cultures every 2–3 days.
11. After specific periods of cultivation, you can process chondrogenic pellets for Alcian 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.

Images of cells in StemPro® Chondrogenesis Differentiation Medium

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.



Related products

Product	Catalog no.
CTS™ StemPRO® MSC SFM	A10332
StemPRO® Human Adipose-Derived Stem Cell Kit	R7788
StemPRO® Adipogenesis Differentiation Kit	A10070-01
StemPRO® Osteogenesis Differentiation Kit	A10072-01
MesenPRO RS™ Medium	12746
DMEM low glucose	11054
FBS, MSC-Qualified (non-US)	12662
GlutaMAX™-I	35050
CELLstart™	A10142
Gentamicin reagent (10 mg/mL)	15710
TrypLE™ Express	12604
DPBS without Ca^{++} and Mg^{++}	14190
Collagenase Type II	17101
Mouse anti-Aggrecan	AHP00
Mouse anti-Osteonectin/SPARC	33-5500
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**.

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**