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StemPro® Osteogenesis Differentiation Kit

Description

The StemPro® Osteogenesis Differentiation Kit has been developed for the osteogenic differentiation of mesenchymal stem cells (MSCs) in tissue-culture vessels. The kit contains all reagents required for inducing MSCs to be committed to the osteogenesis pathway and generate osteocytes. Using the StemPro® Osteogenesis 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 osteocytes.

Product	Catalog no.	Amount	Storage	Shelf life*
StemPro® Osteogenesis Differentiation Kit Contains:	A10072-01	1 kit	—	—
StemPro® Osteocyte/Chondrocyte Differentiation Basal Medium	A10069-01	100 mL	2°C to 8°C; Protect from light	12 months
StemPro® Osteogenesis Supplement	A10066-01	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® Osteogenesis Supplement is supplied frozen. Thaw prior to use, as described in **Prepare media**.
- Thawed StemPro® Osteogenesis Supplement is stable up to at least one month at 2°C to 8°C. Supplement can be refrozen in aliquots and stored at –20°C to –5°C. *Avoid multiple freeze thaw cycles* of supplement.
- Store prepared Complete StemPro® Osteogenesis 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.

Prepare media

Complete Osteogenesis 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.

Osteogenesis Differentiation Medium	Concentration	Volume
StemPro® Osteocyte/Chondrocyte Differentiation Basal Medium	1X	90 mL
StemPro® Osteogenesis 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® Osteogenesis 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, or 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 osteogenesis 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.
- Confluency: Expanding MSCs in growth medium for 2–4 days before refeeding with Complete Osteogenesis Differentiation Medium can enhance osteogenesis.

Osteogenesis 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 5×10^3 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 Osteogenesis Differentiation Medium and continue incubation. MSCs will continue to expand as they differentiate under osteogenic conditions. Refeed cultures every 3–4 days.
11. After specific periods of cultivation, you can process osteogenic cultures for alkaline phosphatase staining (7 –14 days) or Alizarin Red S staining (>21 days; see the following section for method), gene expression analysis, or protein detection.

Alizarin Red S stain analysis

1. After 21 days or longer under differentiating condition, remove media from 12-well plate and rinse once with DPBS. Fix cells with 4% formaldehyde solution for 30 minutes.
2. After fixation, rinse wells twice with distilled water and stain cells with 2% Alizarin Red S solution (pH 4.2) for 2–3 minutes.
3. Rinse wells three times with distilled water, visualize under light microscope and capture images for qualitative or quantitative analysis.

Images of cells in StemPro® Osteogenesis Differentiation Medium

Figure 1 Analysis of MSCs cultured in StemPro® Osteogenesis Differentiation Medium demonstrated differentiation into osteogenic lineages by alkaline phosphatase staining and Alizarin Red S staining.

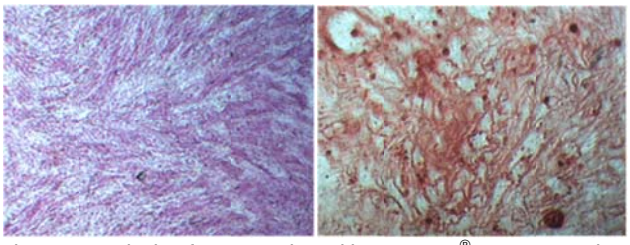
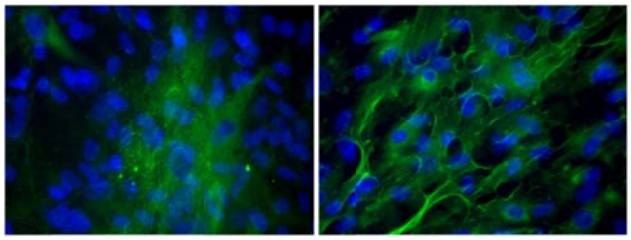


Figure 2 Analysis of MSCs cultured in StemPro® Osteogenesis Differentiation Medium demonstrated differentiation into osteogenic lineages by bone sialoprotein and osteopontin immunostaining.



Related products

Product	Catalog no.
CTS™ StemPRO® MSC SFM	A10332
StemPRO® Human Adipose-Derived Stem Cell Kit	R7788
StemPRO® Adipogenesis Differentiation Kit	A10070-01
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
Mouse anti-Osteocalcin	33-5700
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	Manufacturer	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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