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# STEMPRO® Osteogenesis Differentiation Kit

## Introduction

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 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.

| Description | Cat. no. | Size | Storage | Shelf Life |
|---|---|---|---|---|
| STEMPRO® Osteogenesis Differentiation Kit | A10072-01 | 1 kit | | — |
| Contains: | | | | |
| STEMPRO® Osteocyte/Chondrocyte Differentiation Basal Medium | A10069-01 | 100 mL | 2 to 8°C (protect from light) | 12 months |
| STEMPRO® Osteogenesis Supplement | A10066-01 | 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® Osteogenesis 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 osteogenic lineage.
- Demonstrated to robustly induce osteogenesis in adipose tissue-derived stem cells (STEMPRO® Human Adipose-Derived Stem Cell Kit, Cat. nos. R7788-110 and R7788-115).
- Classical staining methods demonstrate differentiation of MSCs into osteoblasts and osteocytes (Figure 1).
- Immunocytochemistry methods demonstrate expression of relevant osteocyte biomarkers (Figure 2).

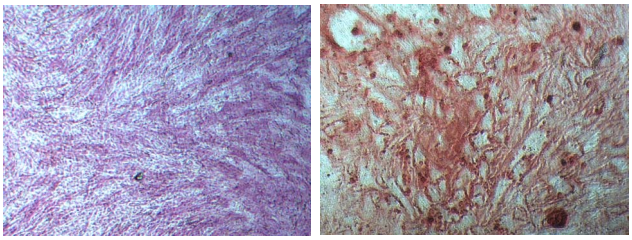

**Figure 1:** Analysis of MSCs cultured in STEMPRO® Osteogenesis Differentiation Medium demonstrated differentiation into osteogenic lineages by alkaline phosphatase staining and Alizarin Red S staining.

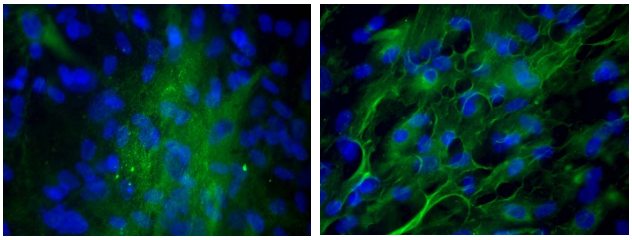

**Figure 2:** Analysis of MSCs cultured in STEMPRO® Osteogenesis Differentiation Medium demonstrated differentiation into osteogenic lineages by bone sialoprotein and osteopontin immunostaining.

## Storage and Handling

- STEMPRO® Osteogenesis Supplement is supplied frozen. Thaw prior to use, as described in **Media Preparation**, next page.
- Thawed STEMPRO® Osteogenesis 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® Osteogenesis 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 Osteogenesis Differentiation

To obtain optimal osteogenic differentiation with STEMPRO® Osteogenesis 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 $3 \times 10^3$ to $5 \times 10^3$ viable cells/cm$^2$ with MesenPRO RS™ Medium or $1 \times 10^4$ viable cells/cm$^2$ with STEMPRO® MSC SFM.
- **Confluency:** Expanding MSCs in growth medium for 2 to 4 days before refeeding with Complete Osteogenesis Differentiation Medium can enhance osteogenesis.

## 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 Osteogenesis Culture

**Media:** StemPro® Osteogenesis 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, or 75-$cm^2$ tissue-culture flasks

## Media Preparation

**Complete Osteogenesis 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.

| Osteogenesis Differentiation Medium | Final Conc. | For 100 mL |
|---|---|---|
| StemPro® Osteocyte/Chondrocyte Differentiation Basal Medium | 1X | 90 mL |
| StemPro® Osteogenesis Supplement | 1X | 10 mL |
| Gentamicin (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 |

## 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 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 an appropriate volume of pre-warmed MSC Growth Medium (see **Media Preparation**).
8. Seed MSCs into culture vessels at 5 × $10^3$ cells/$cm^2$. For classical stain differentiation assays, 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 Complete Osteogenesis Differentiation Medium and continue incubation. MSCs will continue to expand as they differentiate under osteogenic conditions. Refeed cultures every 3 to 4 days.
11. After specific periods of cultivation, osteogenic cultures can be processed for alkaline phosphatase staining (7 to 14 days) or Alizarin Red S staining (>21 days; see below 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 to 3 minutes.
3. Rinse wells three times with distilled water, visualize under light 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® Adipogenesis Differentiation Kit | 1 kit | A10070-01 |
| MesenPRO RS™ Medium | 500 mL | 12746 |
| FBS, MSC-Qualified (non-US) | 100 mL | 12662 |
| GlutaMAX™-I | 100 mL | 35050 |
| Gentamicin (10 mg/mL) | 10 mL | 15710 |
| TrypLE™ Express | 100 mL | 12604 |
| DPBS without $Ca^{++}$ and $Mg^{++}$ | 500 mL | 14190 |
| Mouse anti-Osteocalcin | 100 µg | 33-5700 |
| Rabbit anti-Osteopontin | 100 µg | 42-7701 |
| 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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## Technical Support

Worldwide email: techsupport@invitrogen.com. Toll-free U.S. phone support: 1 800 955 6288. For additional country-specific support, visit our website at www.invitrogen.com\contacts.