PRIME-XV® Osteogenic Differentiation SFM

PRIME-XV® Osteogenic Differentiation SFM (Serum-Free Media) is a serum-free complete medium optimized for the differentiation of human MSCs (Mesenchymal Stem Cells). This product does not contain antibiotics.

Catalog #	Product	Size
91132	PRIME-XV Osteogenic Differentiation SFM	100 mL liquid

Intended Use

PRIME-XV Osteogenic Differentiation SFM is intended for use in the osteogenic differentiation of human MSCs. It can also be used to induce osteogenic differentiation of human amniotic fluid stem cells (AFSCs). This medium is ready to use and can be supplemented with additional cytokine/ growth factors for desired applications.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis which is available at www.irvinesci.com or upon request.

Shipping

This product is shipped with dry ice. Upon receipt, store immediately at the temperature recommended below.

Storage Instructions and Stability

Upon receipt, store the medium at or below -10°C in a manual defrost freezer. Unopened medium is stable for 24 months from date of manufacture, as indicated on label, when stored at or below -10°C in a manual defrost freezer. PRIME-XV Osteogenic Differentiation SFM can be aliquoted and stored at or below -10°C in a manual defrost freezer for up to 3 months. When ready to use, thaw this medium overnight at 2-8°C in the dark. PRIME-XV Osteogenic Differentiation SFM should be used within one week when stored at 2-8°C and protected from light. Not validated for use beyond the unopened expiry shelf life. Repeated freeze thaw cycles should be avoided.

Precautions and Warnings

For research use or further manufacturing use only. Not for injection or diagnostic procedures. This reagent should not be used beyond the expiration date. Results may vary due to variations among human MSCs derived from different donors.

This product contains components derived from human plasma, which has been tested and found negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). However, the medium should be handled as if potentially infectious. Safe laboratory procedures should be followed and protective clothing should be worn when handing this medium. The acute and chronic effects of over-exposure to this medium are unknown.

Directions for Use

The following protocol is optimized for osteogenic differentiation of human MSCs derived from adipose tissue in two dimensional culture vessels.

Preparation of Culture Plates for Osteogenic Differentiation

For monolayer culture of neuronal cells, culture vessels must be pre-coated with substrate for cell attachment. Poly-D Lysine (PDL) (EMD Millipore, Catalog #A-003-E) is recommended.

Note: To induce osteogenic differentiation of MSCs in PRIME-XV Osteogenic Differentiation SFM (Irvine Scientific, Catalog #91132), tissue culture plates or flasks are suggested to be pre-coated with substrate for better cell attachment and preventing cell detachment during differentiation. It is highly recommended that PRIME-XV MatrIS F (Irvine Scientific, Catalog #31001) or PRIME-XV Human Fibronectin (Irvine Scientific, Catalog #31002) be used to coat culture surfaces for better differentiation. Other attachment substrates may be used and must be validated by end-user for optimal performance.

1. Gently add PRIME-XV MatrIS F in PBS (Irvine Scientific, Catalog #9236) to a final concentration of 1 µg/ mL.
2. Add 0.5 mL of diluted coating solution to each well in a 24-well plate.
3. Incubate culture vessels at room temperature for 3 hours or overnight at 2–8°C. The culture vessel must be sealed with Parafilm® to avoid drying if stored at 2–8°C overnight. It is recommended to coat culture vessels the day of use or the day before use.
4. Aspirate out and discard diluted coating solution from culture vessels before the addition of cells.

Osteogenesis Culture Protocol

1. Pre-coat tissue culture plates with 1µg/ mL of PRIME-XV MatrIS F or PRIME-XV Human Fibronectin (See Preparation of Culture Plates for Osteogenic Differentiation section).
2. Seed human MSCs with 8.0 x 10^3 cells in 0.5 mL pre-warmed PRIME-XV MSC Expansion SFM (Irvine Scientific, Catalog #31000) per well of a 24-well plate. Expand cells until an optimal cell density between 50–70% confluence is reached before inducing osteogenesis. Expansion usually takes 1–3 days.
3. When cells reach 50-70% confluence, replace the PRIME-XV MSC Expansion SFM in each well with 0.5mL pre-warmed PRIME-XV Osteogenic Differentiation SFM to initiate differentiation.
4. Incubate at 37°C, 5% CO_2.
5. Remove and discard spent media every 2 days. Feed cells with 0.5mL pre-warmed PRIME-XV Osteogenic Differentiation SFM per well.
6. Approximately 14–21 days under differentiation condition, cells may be fixed for Alizarin Red S staining or processed for immunostaining.

Alizarin Red S Stain Analysis

1. After 14–21 days under differentiation culture, aspirate media from 24-well plates and rinse wells once with PBS without calcium and magnesium (Irvine Scientific, Catalog #9240).
2. Fix cells with 4% formaldehyde solution for 30 minutes.
3. After fixation, rinse wells twice with distilled water and stain cells with 2% Alizarin Red S Solution at 500 µL/well, and incubate at 37°C, 5% CO_2 for 10–20 minutes.
4. Aspirate Alizarin Red S Solution and rinse wells twice with distilled water, visualize under light microscope and capture images.

Data

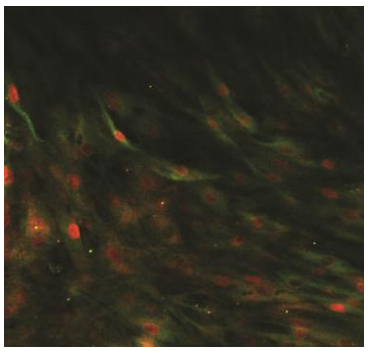

Figure 1. Human adipose-derived MSCs cultured in PRIME-XV MSC Expansion SFM and differentiated into osteocytes using PRIME-XV Osteogenic Differentiation SFM show robust expression of OSTEOCALCIN (green) and RUNX2 (red) based on immunostaining after 2–3 weeks of osteogenic induction. Confocal image was taken at 20X magnification.

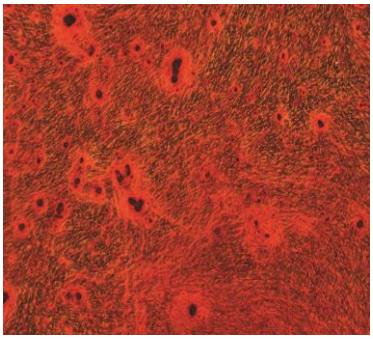

Figure 2. Alizarin Red S detection of Ca^{2+} deposition indicates efficient differentiation of adipose-derived MSCs into matrix-forming osteocytes in PRIME-XV Osteogenic Differentiation SFM after 2–3 weeks of osteogenic induction. Image was taken at 10X magnification.

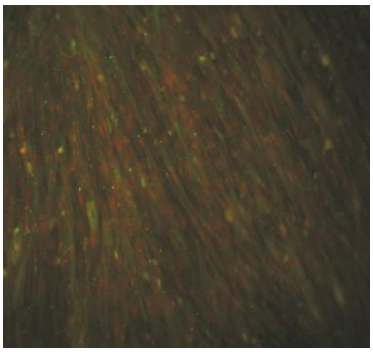

Figure 3. Human AFSCs differentiated for 2−3 weeks in PRIME-XV Osteogenic Differentiation SFM show robust expression of OSTEOCALCIN (green) and RUNX2 (red) based on immunostaining.

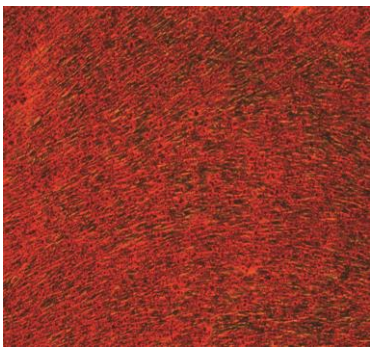

Figure 4. Human AFSCs differentiated for 2–3 weeks in PRIME-XV Osteogenic Differentiation SFM show the presence of osteocytes based on Alizarin Red S staining.

Related Products

Catalog #	Product	Size
91135	PRIME-XV MSC Expansion SFM	250 mL liquid
91149	PRIME-XV MSC Expansion XSFM	250 mL and 1 L liquid
91137	PRIME-XV Adipogenic Differentiation SFM	100 mL liquid
91138	PRIME-XV Chondrogenic Differentiation XSFM	100 mL liquid
31002	PRIME-XV Human Fibronectin	1 mg liquid
91140	PRIME-XV MSC FreezIS DMSO-Free	10 mL and 100 mL liquid

Technical

CONTACT US

For more information or assistance contact Customer Service at:

- Email: tmrequest@irvinesci.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificate of Analysis (CoA) (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

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