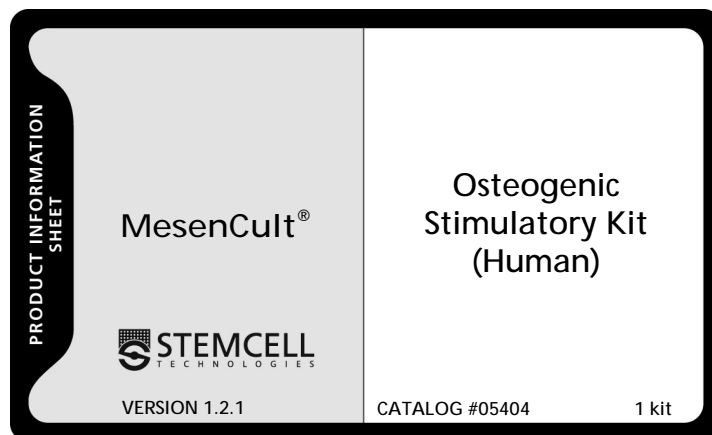


PRODUCT DESCRIPTION

The Osteogenic Stimulatory Kit (Human) is specifically formulated for the *in vitro* differentiation of human mesenchymal progenitor cells into osteogenic progenitors. The kit can also be used for the detection and enumeration of human osteogenic progenitors using the colony-forming unit - osteoblast (CFU-O) assay. This kit can also support CFU-O assays using rat bone marrow cells.



COMPONENTS

This kit contains serum and proprietary supplements pretested and selected for the ability to optimally differentiate human mesenchymal cells into osteogenic progenitors in culture.

This product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

Components have been aseptically manufactured using tightly controlled processes and are sterility tested.

Table 1. Component storage, stability and usage.

CATALOG #	PRODUCT NAME	UNIT SIZE	STORAGE AND STABILITY	USAGE
05401	MesenCult® MSC Basal Medium (Human)	450 mL	Stable at 2 - 8°C for 1 year from date of manufacture as indicated on label.	<ul style="list-style-type: none">• Aliquot into 10 x 45 mL• Store at 2 - 8°C
05405	Osteogenic Stimulatory Supplements (Human)	80 mL	Stable at -20°C until expiry date as indicated on label.	<ul style="list-style-type: none">• Use at 15% final volume• Aliquot into 10 x 8 mL• Store at -20°C
05406	β-Glycerophosphate (1M)	10 mL	Stable at -20°C until expiry date as indicated on label.	<ul style="list-style-type: none">• Use at 3.5 mM (human) or 5.0 mM (rat) final concentration• Aliquot into 10 x 1 mL• Store at -20°C
05407	Dexamethasone	1 mg	Powder stable at 2 - 8°C until expiry date as indicated on label.	<ul style="list-style-type: none">• Use at 10⁻⁸ M final concentration• To prepare stock solution of 10⁻⁴ M, dissolve powder in small volume of absolute ethanol and then add ethanol to a final volume of 25.5 mL• Aliquot into multiple 500 µL vials• Store at -20°C
07157	Ascorbic Acid	100 mg	Powder stable at room temperature (15 - 25°C).	<ul style="list-style-type: none">• Use at 50 µg/mL final concentration• To prepare stock solution of 10 mg/mL, dissolve powder in 10 mL MesenCult® Basal Medium• Aliquot into 10 x 1 mL• Store at -20°C

DIRECTIONS FOR USE

Complete MesenCult® Osteogenic Medium (Human) should be prepared in volumes that can be used within 1 week. To facilitate this, reagents should be aliquoted and stored upon arrival as described in Table 1. Instructions for preparation of 50 mL Complete MesenCult® Osteogenic Medium are provided.

Preparation of Complete MesenCult® Osteogenic Medium:

To prepare 50 mL Complete MesenCult® Osteogenic Medium, pipette 42.5 mL MesenCult® MSC Basal Medium into a 50 mL conical tube and add the following:

- 7.5 mL Osteogenic Supplements
- 5 µL Dexamethasone (10⁻⁴ M stock solution)
- 250 µL Ascorbic acid (10 mg/mL stock solution)
- 175 µL 1M β-Glycerophosphate* (refer to note on following page)



* Note: **β -Glycerophosphate is not added to medium at initiation of the assay.** Typically, β -Glycerophosphate is only added after evidence, by phase microscopy, of cell multilayering. Complete MesenCult® Osteogenic Medium also supports the proliferation of rat osteogenic cells (for rat cells the optimal concentration of β -Glycerophosphate is 5 mM).

Antibiotics and anti-mycotics may be added at the researchers' discretion.

For complete instructions on culturing human mesenchymal cells, refer to the Technical Manual for Enumeration, Expansion, and Differentiation of Human Mesenchymal Progenitor Cells using MesenCult® (Manual Catalog #28453) available on our website at www.stemcell.com/technical/manuals.aspx or contact us to request a copy.

Culture procedure:

Note: If unprocessed bone marrow cells are available, dilute bone marrow 1:3 with PBS + 2% FBS and begin at step 6.

There are many protocols in the literature that describe the development of osteogenic cells from bone marrow or cultured mesenchymal cells.¹⁻⁵ Protocols vary slightly in concentrations of reagents used. The following protocol is one example of a method that supports growth of osteogenic cells from human bone marrow.

1. Prepare cancellous bone fragments by mincing bone into very small pieces (1 - 3 mm in size).
2. Flush fragments with 20 - 30 mL PBS (Catalog #37350) and then vortex fragments with another 20 - 30 mL PBS.
The fragments should appear almost white at this stage.
3. Pass cell suspension through a 70 μ m cell strainer (BD Catalog #352350) to remove bone fragments.
4. Centrifuge cells at 400 x g for 15 minutes.
5. Discard supernatant and resuspend cells in PBS.
6. Place cells on Ficoll-Paque™ PLUS (Catalog #07057) and centrifuge at 400 x g for 25 minutes with the brake set to the 'off' position.
7. Remove the cells at the interface and resuspend cells in Complete MesenCult® Osteogenic Medium (**without β -Glycerophosphate**).
8. Seed cells in tissue culture-treated flasks or plates at a concentration of 1 - 2 x 10⁵ cells per cm².
9. Replenish culture medium after 5 days by removing medium (and non-adherent cells). These cells and the medium can be discarded. Replenish cultures with fresh Complete MesenCult® Osteogenic Medium (without β -Glycerophosphate), **unless cell multilayering has been noted.**
Cell multilayering is the layering of cells on top of each other, forming a matrix, as opposed to growing in a planar manner. Multilayering is indicative of the beginning of bone generation.
10. Once cell multilayering has been observed, supplement Complete MesenCult® Osteogenic Medium with β -Glycerophosphate as directed. Continue to replenish cultures with β -Glycerophosphate-containing medium every 2 - 3 days for a minimum of 3 weeks (for rat cultures) or 5 weeks (for human cultures).
11. Osteogenic cells may be detected by tetracycline labeling² or von Kossa staining. Cultures may be maintained for extended time periods (>8 weeks) for other types of studies.

Ficoll-Paque™ PLUS is a registered trademark of GE Healthcare LTD

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2. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal cells. *Science* 284: 143-147, 1999
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