BI
Biological Industries

MSCgo™ Osteogenic XF

Complete, ready-to-use, serum-free, xeno-free media for the direct differentiation of human mesenchymal stem cells into osteoblasts

Instructions for Use

MSCgo™ Osteogenic XF:
a complete ready to use medium (2-8°C)
05-440-1B 100 ml

MSCgo™ Rapid Osteogenic XF:
a complete ready to use medium (2-8°C)
05-442-1B 100 ml

Product Description

MSCgo™ Osteogenic XF and **MSCgo™ Rapid Osteogenic XF** are a serum-free (SF), xeno-free (XF) complete ready to use formulations, developed for the differentiation of human Mesenchymal stem cells (hMSC) into mature osteocytes.

The media are suitable for variety sources of hMSC (e.g. bone marrow, adipose tissue and umbilical cord tissue; hMSC-BM, hMSC-AT, hMSC-CT).

MSCgo™ Rapid Osteogenic XF will lead to faster osteogenesis (less than 10 days) in comparison to the MSCgo™ Osteogenic XF (14-21days).

Notes:
No additional additives are required for the complete, ready-to-use media.
Contain L-alanine L-glutamine.
Do not contain antibiotics.

Product Use

For human ex vivo tissue and cell culture processing applications. It is not approved for human or animal use, or for application of in vitro diagnostic procedures.

Oseogenesis Results

Osteogenic differentiation of hMSC results in the formation of mineralized culture with calcified nodules and calcium secretion that can be detected with Alizarin Red S (ARS) staining.
The ARS is used to stain calcium deposits formation which are an indication of mature osteocytes.
The amount of calcified nodules formation and calcium secretion can be varying using different hMSC (e.g. types, age and passage).

Required Materials for Osteogenic Assay

- **MSCgo™ Osteogenic XF** BI; 05-440-1 **or**
  MSCgo™ Rapid Osteogenic XF BI; 05-442-1
- MSC NutriStem® XF : BI; 05-200-1 and 05-201-1
- MSC Attachment solution XF : BI; 05-752-1
- Optional- Alizarin Red S (ARS).

Precautions and Disclaimer

1. Do not use if a visible precipitate is observed in the medium..
2. Do not use the media beyond the expiration date indicated on the product label.

Note: *Always use proper aseptic technique and work in a laminar flow hood*

Culture of Excellence

Osteogenic Differentiation

Note: *When handling biohazard materials such as human cells, appropriate safety procedures should always be used and protective clothing and gloves should be worn.*

1. **Initial Seeding:** seed 6x10^4 cells/well in 24-well plate (3x10^4/cm^2) using 0.5ml/well of MSC NutriStem® XF, on pre-coated plates (using MSC attachment solution BI; 05-752-1, diluted 1:100 in DPBS).

   Note: *For any other cultureware, the appropriate volume should be adjusted.*

2. Incubate the cells in CO_2 incubator (37°C, 5% CO_2).
3. **Initial of differentiation:** after 24hr from cell's seeding, ensure that the cells reach about 80% confluence and change medium to differentiation medium (0.5ml/well; 24w/p).

   Note: *If the cells confluence is <80% continue culturing in MSC NutriStem® XF for one more day.*

4. Incubate the cells with MSCgo™ Osteogenesis XF (BI; 05-440-1) or MSCgo Rapid Osteogenic XF (BI; 05-442-1) for 10-21 days in incubator (37°C, 5% CO_2). Change the medium every 2-3 days.

   Note: *The longer the incubation time, the more mineralized culture will be obtained (as indicated by higher intensity of ARS staining).*

5. **Evaluate of the osteogenesis.** 2% ARS solution can be used for the osteogenesis evaluation.

   For the staining procedure follow the instructions ahead.

ARS Staining Protocol (Optional)

Preparation of 2% ARS Solution

Note: *the solution should be freshly prepared or required PH calibration to 4.1-4.3.*

1. Dissolve 2gr of ARS in 100ml DDW.
2. Adapt PH to 4.1-4.3 (with 0.1N HCl or 0.5% v/v NH_3).
3. Mix well and filter through a 0.45 micron CA syringe filter (MINISART 16555).
4. The solution is stable for one year (2-8°C).
5. Always before use, check the PH and adapt to 4.1-4.3 if necessary (with 0.1N HCl or 0.5% v/v NH_3).

Staining Procedure

1. Carefully remove the medium and gentle wash once with DPBS BI; 02-023-1 (1ml/well; 24w/p).
2. Fixation: carefully remove DPBS and add cold Ethanol (EtOH) 70% (1ml/well; 24w/p).
3. Incubate at room temperature for 30-60 minutes.
4. Remove Ethanol (EtOH) and wash 3 times with DDW (1ml/well; 24w/p).
5. Remove DDW and add 1ml of 2% ARS solution to each well.
6. Incubate at room temperature for 30 min.-1hr.
7. Remove ARS solution and wash 4 times with DDW (1ml/well; 24w/p).

   Note: *calcium secreted from cells will wash out. Nodular structures will remain with positive staining for calcium content.*

8. Add DDW to each well (1ml/well; 24w/p) to prevent the cells from drying.
9. The plate is now ready for visual inspection, image acquisition and evaluation of osteogenesis.

   Note: *Osteocytes containing calcium deposits will be stained orange red by the ARS.*

Semi-Quantification of ARS Staining (Optional)

Semi-quantification of the mineralization can be performed by ARS elution.

1. For ARS elution add 10% (w/v in DDW) of CPC (CetylPyridinium Chloride) 0.5ml/well.
2. Incubate at room temperature for 1hr.
3. Read the absorbance (O.D.) at 550nm (10% CPC serves as blank), (150µl/well; 96w/p).

Quality Control

MSCgo™ Osteogenesis XF (BI; 05-440-1) and MSCgo™ Rapid Osteogenic XF (BI; 05-442-1) performance is tested for optimal differentiation of hMSC into osteocytes. Additional tests are: pH, osmolality, endotoxins and sterility tests.

Biological Industries Israel Beit Haemek Ltd.
Kibbutz Beit Haemek 2511500, Israel
T.+972.4.9960595 F.+972.4.9968896
Email: info@bioind.com
www.bioind.com