

Instructions for Use

MSCgo™ Osteogenic XF

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




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SARTORIUS

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

	MSCgo™ Osteogenic XF Medium	MSCgo™ Rapid Osteogenic XF Medium
REF	05-440-1	05-442-1
	2-8°C	2-8°C

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1 Product Description

MSCgo™ Osteogenic XF and MSCgo™ Rapid Osteogenic XF are 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 hMSC from a variety of sources (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 - 21 days).

Oseogenesis Results

Osteogenic differentiation of hMSC results in the formation of mineralized culture with calcified nodules and calcium secretion that can be visually 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 vary using different hMSC (e.g. source, age and passage).

2 Precaution 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.
3. Always use proper aseptic technique and work in a laminar flow hood.

3 Storage & Stability

- Store MSCgo™ Osteogenic XF and MSCgo™ Rapid Osteogenic XF at 2-8°C.
- Protect the media from light.
- Shelf life: refer to product label for expiration date.

4 Features

- Serum-free, xeno-free solution
- Complete, ready-to-use differentiation medium
- Reliable differentiation to mature osteocytes
- Contains stable L-alanyl-L-glutamine
- Does not contain antibiotics

5 Required Materials for Osteogenic Assay

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

6 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 6×10^4 cells/well in 24-well plate ($3 \times 10^4/\text{cm}^2$) using 0.5 mL/well of MSC NutriStem® XF, on pre-coated plates (using MSC Attachment Solution 05-752-1, diluted 1:100 in DPBS).

NOTE For any other cultureware, the appropriate volume should be adjusted.

2. Incubate the cells in a CO₂ incubator (37°C, 5% CO₂).
3. Initial of differentiation: after 24 hr from cell's seeding, ensure that the cells reach about 80% confluence and change medium to differentiation medium (0.5 mL/well; 24 w/p).

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

4. Incubate the cells with MSCgo™ Osteogenic XF 05-440-1 or MSCgo Rapid Osteogenic XF 05-442-1 for 10 - 21 days in incubator (37°C, 5% CO₂). 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.

7 ARS Staining Protocol (Optional)

Preparation of 2% ARS Solution

NOTE The PH of the solution must be adjusted to 4.1-4.3.

1. Dissolve 2 gr of ARS in 100 mL DDW.
2. Adjust PH to 4.1-4.3 (with 0.1N HCl or 0.5% v/v NH₃).
3. Mix well and filter through a 0.45 µm CA syringe filter (Minisart® 16555).
4. The solution is stable for one year (2-8°C).
5. Always before use, check the PH and adjust to 4.1-4.3 if necessary (with 0.1N HCl or 0.5% v/v NH₃).

Staining Procedure

1. Carefully remove the medium and gently wash once with DPBS (Cat. No. 02-023-1); (1 mL/well; 24 w/p).
2. Fixation: carefully remove DPBS and add cold Ethanol (EtOH) 70% (1 mL/well; 24 w/p).
3. Incubate at room temperature for 30-60 minutes.
4. Remove Ethanol (EtOH) and wash 3 times with DDW (1 mL/well; 24 w/p).
5. Remove DDW and add 1 mL of 2% ARS solution to each well.
6. Incubate at room temperature for 30 min. - 1 hr.
7. Remove ARS solution and wash 4 times with DDW (1 mL/well; 24 w/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 (1 mL/well; 24 w/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) 0.5 mL/well.
2. Incubate at room temperature for 1 hr.
3. Read the absorbance (O.D.) at 550 nm (10% CPC serves as blank), (150 μ L/well; 96 w/p).

8 Quality Control

MSCgo™ Osteogenesis XF and MSCgo™ Rapid Osteogenesis XF performance is tested for optimal differentiation of hMSC into osteocytes. Additional tests are: pH, osmolality, endotoxins and sterility tests.

9 Quality Assurance

- USP <1043> ancillary product.
- Manufactured under ISO 13485 QMS and in compliance with applicable cGMP guidelines.
- Manufactured under controlled environments and processes in accordance with:
 1. ISO 13408 – Aseptic Processing of Health Care Products;
 2. ISO 14644 – Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones.

9.1 Product Label Symbols

REF

Indicates the manufacturer's catalogue number so that the product can be identified.

LOT

Indicates the manufacturer's batch code so that the batch or lot can be identified.

NOTE Synonyms for batch code are lot number and batch number.



Indicates the date after which the product is not to be used.



Indicates the temperature limits to which the product can be safely exposed.

STERILE A

Indicates a product that has been manufactured using accepted aseptic techniques.

10 Related Products

Product	Cat. No.
Dulbecco's PBS (w/o Ca & Mg)	02-023-1
MSC NutriStem® XF Medium	05-200-1
MSC NutriStem® XF Supplement Mix	05-201-1

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Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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