

Instructions for Use

MSCgo™ Chondrogenic XF

Serum-free, xeno-free medium for the direct differentiation of human mesenchymal stem cells into chondrocytes




2740213-000



SARTORIUS

Serum-free, xeno-free medium for the direct differentiation of human mesenchymal stem cells into chondrocytes

	MSCgo™ Chondrogenic XF Basal Medium	MSCgo™ Chondrogenic XF Supplement Mix
REF	05-220-1	05-221-1
	2-8°C	-20 to -10°C

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1 Product Description

MSCgo™ Chondrogenic XF is a serum-free (SF), xeno-free (XF) medium developed for the differentiation of human Mesenchymal Stem Cells (hMSC) into mature chondrocytes.

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

Chondrogenesis Results

Chondrogenic differentiation of hMSC in 3D spheroid culture results in the formation of cartilage with a typical extracellular matrix rich of Aggrecan. Aggrecan is a proteoglycan that can be used as an indicator for cartilage formation and can be detected with Alcian Blue, a dark-blue copper-containing dye. Alcain Blue staining is an indication of mature chondrocytes. The staining intensity 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™ Chondrogenic XF Basal Medium at 2–8°C.
- Store MSCgo™ Chondrogenic XF Supplement Mix at -20 to -10°C.
- Protect the medium and supplement from light.
- Shelf life: Refer to product label for expiration date.

4 Features

- Serum-free, xeno-free medium.
- All required growth factors and supplements included.
- Reliable differentiation to mature chondrocytes.
- Each lot is application tested.
- Does not contain antibiotics.

5 Complete Ready-to-Use Medium Preparation

1. Thaw MSCgo™ Chondrogenic XF supplement mix (05-221-1D) at room temperature (RT).
2. Dilute the supplement mix 1:10 in the MSCgo™ Chondrogenic XF basal medium (05-220-1B). (e.g. 10 mL of supplement mix + 90 mL of basal medium). By adding the supplement mix into the basal medium a complete ready to use medium is achieved.
3. The complete medium is stable for 1 month at 2–8°C.

NOTE No additional additives are required for the complete, ready-to-use medium.

6 Required Materials for Chondrogenic Assay

- MSCgo™ Chondrogenic XF Medium and Supplement: 05-220-1 and 05-221-1.
- 96-well U-bottom, non-tissue culture treated plate (for suspension).
- MSC NutriStem® XF Medium and Supplement: 05-200-1 and 05-201-1.
- Optional: Alcian Blue 8 GX.

7 Chondrogenic Differentiation Assay

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 of hMSC for 3D spheroid culture: Seed 1×10^5 cells/well in a 96-well U-bottom culture plate using 100 μL of MSC NutriStem® XF, without pre-coating procedure.

NOTE A micromass culture technique is also an option and will advance the spontaneously spheroid's formation: seed 10 μL of 1×10^7 cells/ml into the center of the well (final of 1×10^5 cells/well), allow to adhere for 2 hr, then add 0.1 mL/well of medium.

2. Incubate the cells in a CO_2 Incubator (37°C , 5% CO_2). Spheroids will spontaneously form within 24 - 48 hours.
3. Initial of differentiation: after 24 hr from cells seeding change the medium to the complete MSCgo™ Chondrogenic XF Medium (200 μL /well; 96 w/p).
4. Incubate the cells with the complete MSCgo™ Chondrogenic XF Medium for 14 - 21 days in an incubator (37°C , 5% CO_2).

NOTE The longer incubation time, the more mature chondrocytes will be obtained (as indicated by higher intensity of Alcian Blue staining).

5. Change the complete MSCgo™ Chondrogenic XF medium every 3-4 days. (200 µL/well; 96 w/p).

NOTE Be careful not to aspirate the spheroids.

6. Evaluate of chondrogenesis: Alcian Blue staining can be used for the evaluation. Using Alcian Blue staining, the proteoglycan aggrecan, an indicator for cartilage formation, will be dark-blue stained.

8 Alcian Blue Staining Protocol (Optional)

Preparation of 1% Alcian Blue Solution

1. Dissolve 0.2 gr of Alcian Blue 8 GX in 20 mL 0.1N HCl.
2. Mix well and filter through a 0.45 µm syringe filter (Minisart® 16555).
3. The solution is stable for one year (2-8°C).

Staining Procedure

NOTE Be careful not to aspirate the spheroids.

1. Carefully remove the medium and gently wash once with DPBS 02-023-1 (0.2 mL/well; 96 w/p).
2. Fixation: carefully remove DPBS and add 10% Formalin (4% v/v Formaldehyde) to each well (0.2 mL/well; 96 w/p). Incubate at room temperature for 30 - 60 minutes.
3. Remove formalin solution and wash twice with DDW (0.2 mL/well; 96 w/p).
4. Remove DDW and add 0.2 mL of 1% Alcian Blue solution to each well. Incubate at room temperature overnight. Protect from light!
5. Remove staining solution and wash 2-3 times with 0.1N HCl (200 µL/well; 96 w/p).

6. Remove HCL solution and add DDW to each well (0.2 mL/well; 96 w/p).
7. The plate is now ready for visual inspection, image acquisition and evaluation of chondrogenesis.

NOTE Cartilage containing aggrecans stains blue whereas spheroids without aggrecans lose the staining during the washing steps.

Semi-Quantification of Alcian Blue Staining (Optional)

Semi-quantification of aggrecans formation can be performed by Alcian Blue elution.

1. For Alcian Blue elution, add 8M Guanidine HCl solution (GuHCl) (150 μ L/well; 96 w/p).
2. Incubate over night, at 2 - 8°C.
3. Read the absorbance (O.D.) at 600 nm (8M GuHCl serves as blank) (150 μ L/well; 96 w/p).

9 Quality Control

MSCgo™ Chondrogenesis XF performance is tested for differentiation of hMSC into chondrocytes. Additional tests are: pH, osmolality, endotoxins and sterility tests.

10 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.

10.1 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.

11 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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