



# MSCgo™ Chondrogenic XF

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

## Instructions for Use

### Medium Components

Product Description	Storage	Cat. No.	Size
MSCgo™ Chondrogenic XF Basal Medium	2-8°C	05-220-1B	100ml
MSCgo™ Chondrogenic XF Supplement Mix	-20°C	05-221-1D	10ml

### 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 variety sources of hMSC (e.g. bone marrow, adipose tissue and umbilical cord tissue; hMSC-BM, hMSC-AT, hMSC-CT) .

### 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.

### 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. Alcian Blue staining is an indication of mature chondrocytes. The staining intensity can be vary using different hMSC (e.g. types, age and passage).

### 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. 10ml of supplement mix + 90ml 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.

#### Notes:

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

*Does not contain antibiotics.*

### 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*

### Required Materials for Chondrogenic 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.

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

### 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.*

## 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  $1 \times 10^5$  cells/well in 96-well U-bottom culture plate ( $30\text{-}40 \times 10^4$  cells/cm<sup>2</sup>) using 100  $\mu$ l of MSC NutriStem<sup>®</sup> XF, **without pre-coating procedure.**

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

2. Incubate the cells in CO<sub>2</sub> Incubator (37°C, 5% CO<sub>2</sub>).  
**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<sup>™</sup> Chondrogenic XF medium (200  $\mu$ l/well; 96w/p).
4. Incubate the cells with the complete MSCgo<sup>™</sup> Chondrogenic XF medium for 14-21 days in incubator (37°C, 5% CO<sub>2</sub>).

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

5. Change the complete MSCgo<sup>™</sup> Chondrogenic XF medium every 3-4 days (200  $\mu$ l/well; 96w/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.

**For the staining procedure follow the instructions ahead.**

## Alcian Blue Staining Protocol (Optional)

### Preparation of 1% Alcian Blue Solution

1. Dissolve 0.2gr of Alcian Blue 8 GX in 20ml 0.1N HCL.
2. Mix well and filter through a 0.45 micron 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 gentle wash once with DPBS BI; 02-023-1 (0.2ml/well; 96w/p).
2. **Fixation:** carefully remove DPBS and add 10% Formalin (4% v/v Formaldehyde) to each well (0.2ml/well; 96w/p). Incubate at room temperature for 30-60 minutes.
3. Remove formalin solution and wash twice with DDW (0.2ml/well; 96w/p).
4. Remove DDW and add 0.2ml 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  $\mu$ l/well; 24w/p).
6. Remove HCL solution and add DDW to each well (0.2ml/well; 96w/p).
7. The plate is now ready for visual inspection, image acquisition and evaluation of chondrogenesis.

**Note:** Cartilage containing aggrecans stays blue whereas spheroids without aggrecans lose the staining during the washing steps.

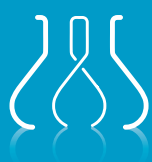
### Semi-Quantification of Alcian Blue Staining (Optional)

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

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

### Quality Control

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



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