Instructions for Use

MSC NutriStem® XF

A Defined, Xeno-Free (XF), Serum-Free (SF) Medium,
Designed to Support the Growth of hMSC

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SARTORIUS

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	MSC NutriStem® XF Basal Medium	MSC NutriStem® XF Supplement Mix	MSC NutriStem® XF Basal Medium, without Phenol Red
REF	05-200-1	05-201-1, 05-213-1	05-202-1, 05-212-1
	2 – 8°C	-10°C to -20°C	2 – 8°C

Contents

1 Product Description and Important Notes

MSC NutriStem® XF Medium is a serum-free, xeno-free medium formulation developed for the growth and expansion of human mesenchymal stem cells after being isolated from a variety of sources (i.e., bone marrow, adipose tissue, umbilical cord tissue and Dental pulp ; BM-hMSC, AT-hMSC, UCT-hMSC, DP-hMSC). No adaptation is required for the transition from serum-containing medium to the MSC NutriStem® XF Medium.

MSC NutriStem® XF Medium is recommended for use with NutriCoat™ Attachment Solution (05-760-1-15) for optimal attachment and spreading of cells.

MSC NutriStem® XF Medium supports long-term growth of hMSC while maintaining their self- renewal and multi-lineage differentiation potential.

For optimal cell passage and long term culture of the cells, it is recommended to use Recombinant Trypsin Solution (03-078-1 or 03-079-1).

Isolation
For the initial isolation of hMSC it is recommended to add 2 – 2.5% human AB serum to the complete medium to facilitate cell's attachment and growth. (The requirement of human AB serum may be varied between different hMSC sources). The pre-coated step with NutriCoat™ Attachment Solution is also required for the initial isolation of hMSC (with and without the addition of human AB serum.)

Alternatives to the pre-coating step
MSC NutriStem® XF complete medium may promotes hMSC proliferation without a pre coating step using advanced surface treated cultureware (e.g. Corning CellBIND).

In addition, MSC NutriStem® XF complete medium supplemented with 5% human Platelet lysate (hPL) may also enable hMSC culturing w/o the pre-coating procedure.

2 Features

- Serum-free (SF), xeno-free (XF) medium: all components are defined and from non-xenogenic origin, including proteins.
- Enables culture of hMSC from different sources.
- Supports long-term growth of hMSC, retaining the fibroblast-like cell structure.
- No background differentiation.
- Maintains hMSC self-renewal and multi-lineage differentiation potential (e.g., osteocytes, adipocytes and chondrocytes).
- Human MSC cultured with MSC NutriStem® XF express high percentage of MSC surface markers and do not express hematopoietic markers.

3 Intended Use and Safety

- For research or further manufacturing use as ancillary material in manufacturing of cell, gene or tissue-based products
- Not intended for human in vivo applications
- Not intended for in vitro diagnostics use or use as a medical device
- Do not use the medium if visible particles and or precipitate are observed.
- Do not use MSC NutriStem® XF Medium beyond the expiration date indicated on the product label.
- Do not use in case of change of color.
- Maintain aseptic work conditions.
- Do not use if there is any package leakage or any exposure to environmental conditions as the sterility of the product might be compromised.
- Please refer to the Safety Data Sheet (SDS) for hazard information.

4 Storage and Stability

Product Description	Storage	Cat. No.	Size
MSC NutriStem® XF Basal Medium	2 – 8°C	05-200-1A	1 × 500 mL
MSC NutriStem® XF Basal Medium	2 – 8°C	05-200-1B	1 × 100 mL
MSC NutriStem® XF Basal Medium, without Phenol Red	2 – 8°C	05-202-1	1 × 500 mL
MSC NutriStem XF Basal Medium (FRP) - UO	2 – 8°C	05-212-1A	1 × 500 mL
MSC NutriStem® XF Supplement Mix	-10°C to -20°C	05-201-1U	1 × 3 mL
MSC NutriStem® XF Supplement Mix	-10°C to -20°C	05-201-1-06	1 × 0.6 mL
MSC NutriStem XF Supplement Mix - UO	-10°C to -20°C	05-213-1U	1 × 3 mL

NOTE

- No additional additives are required for the complete, ready-to-use medium.
- Contains L-glutamine.
- Does not contain antibiotics.
- Protect from direct light.
- Shelf life: Refer to product label for expiration date.c

5 Adaptation of hMSC to MSC NutriStem® XF Medium

hMSC can be transferred directly to MSC NutriStem® XF Medium, without prior adaptation from any other culture media (including serum containing medium).

6 Complete Ready-To-Use Medium Preparation

- The frozen MSC NutriStem® XF Supplement Mix should be thawed at 2 – 8°C or at room temperature. Avoid repeated freeze-thaw cycles (up to two times).
- For a complete medium, aseptically add 3 mL of MSC NutriStem® XF Supplement Mix to 500 mL of MSC NutriStem® XF Basal Medium. (Alternatively, aseptically add 0.6 mL of MSC NutriStem® XF Supplement Mix to 100 mL of MSC NutriStem® XF Basal Medium).
- MSC NutriStem® XF Basal Medium contains L-glutmine.
- Store at 2 – 8°C, protected from light.
- The complete MSC NutriStem® XF Medium is stable at 2 – 8°C for up to 30 days, protected from light.

7 Preparation of Pre-Coated Cultureware with NutriCoat™ Attachment Solution (Cat. No. 05-760-1-15)

Pre-coating Procedure

1. Dilute the solution 1:500 in saline and gently mix using a pipette.
2. Coat the culture dish with the 1:500 solution. The volume should be adequate for covering the surface of the cultureware. Use table 1 for the recommended volumes (e.g. per 1 well of 12 w/p add 2 µL of NutriCoat™ in 1 mL saline).
3. Gently agitate and incubate for 1 hour in a humidified CO_2 incubator (37°C).

   NOTE Longer incubation of coated culture dish (up to 24 hours) will not affect cell attachment and spreading.

4. Wash step is not required.

   NOTE One wash using Dulbecco's PBS (without Ca & Mg) is an option.

5. Prior to cells seeding, carefully discard the solution and add a culture medium.

   NOTE It is critical that the coating does not dry out.

Table 1: Recommended volume for the coating procedure

Cultureware	Surface area cm^2	Volume of the 1:500 diluted solution
96-well	0.34	0.1 mL
24-well	1.9	0.5 mL
12-well	3.9	1 mL

Cultureware	Surface area cm^2	Volume of the 1:500 diluted solution
6-well	9.6	2.5 mL
T25 Flask	25	6.5 mL
T75 Flask	75	19 mL

8 Culturing of hMSC in the complete MSC NutriStem® XF Medium

A. Recovery of Cryopreserved hMSC

1. Pre warm 5 – 10 mL of complete MSC NutriStem® XF Medium in a 50 mL conical tube.
2. Rapidly thaw frozen vial of hMSC in a 37°C water bath, with agitation untill a small amount of ice remains.
3. Slowly add (drop by drop while gently swirling) the cells into the pre-warmed complete MSC NutriStem® XF Medium.
4. Centrifuge cells at 300 – 400 xg for 4 – 5 minutes at room temperature.
5. Remove supernatant and re-suspend cell pellet in 0.5 – 1 mL of complete MSC NutriStem® XF Medium.
6. Perform a viable cell count (e.g., using Trypan Blue Exclusion Assay)
7. Add the desired volume of complete MSC NutriStem® XF Medium.
8. Transfer the cells into NutriCoat™ Attachment Solution pre-coated cultureware (see above). Seeding densities should be calculated (see table 2).
9. Incubate in a humidified CO_2 incubator (37°C).

NOTE
It is possible to avoid the centrifugation step after thawing. In this case skip steps 4 – 5 and transfer the thawed cells (from Step 3) directly into the pre-coated culture flask using NutriCoat™ Attachment Solution (Cat. No. 05-760-1-15) with the required volume of the complete MSC NutriStem® XF Medium, at a ratio of at least 1:10 (for the dilution of the DMSO).

B. Subculturing hMSC
MSC NutriStem® XF Medium was developed for optimal proliferation of hMSC from a variety of sources (BM-hMSC, AT-hMSC, UCT-hMSC). The variety sources and the variability of donors may influence hMSC proliferation rate. For optimal proliferation of hMSC in MSC NutriStem® XF Medium, it is recommended to seed hMSC at a concentration of 6000 – 5000 cell/cm² (Table 2), re-feed cells with fresh warmed complete MSC NutriStem® XF Medium every 2 – 3 days and subculture when the cells reach up to 80% confluence (usually 4 – 3 days post seeding). Avoid overgrown culture, as it leads to cell's maturation and senescence.

Subculturing Protocol
1. Remove culture medium and gently wash once with DPBS w/o Ca, Mg (Cat. No. 02-023-1).
2. For T25 culture flask add 1 – 3 mL of Recombinant Trypsin Solution with or without EDTA (Cat. No. 03-078-1, 03-079-1). (For any other cultureware, the appropriate volume should be adjusted).

   NOTE The more the culture is confluence, the slower the detachment will be and the higher volume is recommended.

3. Incubate for 2 – 10 minutes at room temperature and verify cell detachment using inverted microscope. (Incubation at 37°C will not accelerate detachment). Usually, within 2 – 5 minutes (at R.T.) the cells will dissociate by gently tapping the flask.
4. Following detachment, add 5 – 10 mL of pre-warmed MSC NutriStem® XF. Alternatively use diluted (1:50, in DPBS) Soybean Trypsin Inhibitor (SBTI) (Cat. No. 03-048-1). Collect cell suspension into sterile tube and re-wash the cultureware as necessary to collect the entire cells.
5. Centrifuge cells for 4 – 5 minutes at 300 – 400 xg at room temperature.

Carefully discard the supernatant.

6. Re-suspend cell pellet in minimal volume of pre-warmed complete MSC NutriStem® XF Medium. Take sample volume to perform a viable cell count. For cryopreservation continue with section C.
7. Re-seed cells into pre-coated cultureware (see above). Seeding densities and the required volume of complete MSC NutriStem® XF Medium to be added should be calculated (see Table 2).
8. Incubate in a humidified CO_2 incubator (37°C).
9. Re-feed cells with fresh warmed complete MSC NutriStem® XF Medium every 2 – 3 days.

Table 2: Recommended seeding densities (approximately 5000 – 6000 cells/cm²)

Cultureware	12- well plate	6-well plate	T25-Flask
Surface area cm²	3.9	9.6	25
Volume of complete MSC NutriStem® XF Medium	1 – 2 mL/well	2 – 3 mL/well	5 – 6 mL/T-25
Recommended seeding densities	$1.8 – 2.1 \times 10^4$ cells/well	$4.3 – 5.3 \times 10^4$ cells/well	$11 – 14 \times 10^4$ cells/well

C. Cryopreservation of hMSC

1. Rapidly re-suspend hMSC pellet with cold NutriFreez® D10 Cryopreservation Medium (Cat. No. 05-713-1) (recommended between $0.5 – 1 \times 10^6$ cells/mL, 1 mL/vial).
2. Immediately place the cryovials in appropriate freezing container (e.g., "Mr. Frosty") and place at -80°C for overnight.
3. Transfer the cryovials into liquid nitrogen.

9 Quality Control

MSC NutriStem® XF Medium performance is tested for optimal maintenance and expansion of undifferentiated hMSC, while maintaining their multilineage differentiation potential. Additional tests are: pH, osmolality, endotoxins and sterility tests. For full specifications please check the lot specific Certificate of Analysis (CoA).

10 Quality Assurance

- For in vitro diagnostic use, research use or for use as ancillary material in manufacturing cell, gene and tissue-based products.
- Listed in Europe under CE IVD class I, thus comply with European In-Vitro Diagnostic Devices Directive (98/79/EC) requirements.
- Notified under US FDA IVD part 864.2220 Synthetic cell and tissue culture media and components.
- Manufactured under ISO 13485 QMS and ISO 9001 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.
- Submitted under US FDA MF (Master File) and Health Canada MF (Master File).

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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.
	Indicates the need for the user to consult the instructions for use.

11 Auxiliary Products

Product	Cat. No.
NutriCoat™ Attachment Solution	05-760-1
NutriFreez® D10 Cryopreservation Medium	05-713-1
Recombinant Trypsin Solution	03-078-1
Recombinant Trypsin-EDTA Solution	03-079-1
Soybean Trypsin Inhibitor (SBTI)	03-048-1
Dulbecco's PBS (w/o Ca & Mg)	02-023-1

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

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