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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	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	05-202-1
	2 – 8°C	-10°C to -20°C	2 – 8°C

Contents

1 Product Description and Important Notes 4

2 Features 5

3 Precaution and Disclaimer 5

4 Features 6

5 Adaptation of hMSC to MSC NutriStem® XF Medium 6

6 Complete Ready-To-Use Medium Preparation 7

7 Preparation of Pre-Coated Cultureware with MSC Attachment Solution 7

8 Culturing of hMSC in the complete MSC NutriStem® XF Medium 9

9 Quality Control 11

10 Quality Assurance 12

10.1 Product Label Symbols 13

11 Auxiliary Products 14

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 MSC Attachment Solution (Cat. No. 05-752-1) 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 MSC 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 Medium Components and Storage

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

NOTE

- No additional additives are required for the complete, ready-to-use medium.
- Contains L-glutamine.
- Does not contain antibiotics.
- Components are not sold separately.

3 Precaution and Disclaimer

- Do **not** use if a visible precipitate is observed in the medium.
- Do **not** use MSC NutriStem® XF Medium beyond the expiration date indicated on the product label.
- Please refer to the Safety Data Sheet (SDS) for hazard information.

4 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.

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 MSC Attachment Solution (Cat. No. 05-752-1)

1. Dilute MSC Attachment Solution 1:100 using sterile DPBS (without Ca++ and Mg++ (Cat. No. 02-023-1) and gently mix using a pipette.
2. Add the diluted MSC Attachment Solution to the cultureware. Gently agitate the coated cultureware and verify complete covering of the surface. Use Table 1 for recommended volumes.
3. Incubate the coated cultureware for at least 30 minutes in a humidified CO_2 incubator (37°C).

4. Following 30 minutes incubation:

For immediate use:
- Gently wash the cultureware with DPBS (For T-25 use at least 5 mL).
- Seed cells immediately.

It is critical that the coating does not dry out.

For later use:
- Wrap the coated cultureware with Parafilm® and incubate at 2–8°C. Coated cultureware stored under sterile conditions at 2–8°C are stable for 1 week.
- Gently wash the cultureware with DPBS.
- Seed cells immediately.

It is critical that the coating does not dry out.

Table 1: Recommended volume for the coating pocedure

Cultureware	Surface area cm^2	Volume of 1:100 MSC Attachment Solution
96-well	0.34	0.1 mL
24-well	1.9	0.4 mL
12-well	3.9	0.8 mL
6-well/35 mm ware	9.6	2 mL
6 cm/T25 Flask	25	5 mL
T75 Flask	75	15 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 MSC 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 MSC Attachment Solution (Cat. No. 05-752-1) 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.
- Listed in the US FDA under IVD Class I.
- 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.
- Submitted under US FDA MF (Master File) and Health Canada MF (Master File).

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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.
CE	Indicates that the product meets the requirements of the applicable EC directives
IVD	Indicates a product that is intended to be used as an in vitro diagnostic medical device.
	Indicates the need for the user to consult the instructions for use.

11 Auxiliary Products

Product	Cat. No.
MSC Attachment Solution	05-752-1
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
NutriCoat™ Attachment Solution	05-760-1

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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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