



MSC NutriStem® XF Medium

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

Instructions for Use

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 culture ware (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.

Medium Components

Product Description	Storage	Cat. No.	Size
MSC NutriStem® XF Basal Medium	2-8°C	05-200-1A	1x500 ml
MSC NutriStem® XF Supplement Mix	-5 to -20°C	05-201-1U	1x3 ml
MSC NutriStem® XF Basal Medium	2-8°C	05-200-1B	1x100 ml
MSC NutriStem® XF Supplement Mix	-5 to -20°C	05-201-1-06	1x0.6 ml

Notes:

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

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.

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).

Precautions and Disclaimer

1. Do not use if a visible precipitate is observed in the medium.
2. Do not use MSC NutriStem® XF Medium beyond the expiration date indicated on the product label.

Complete Ready-To-Use Medium Preparation

The frozen MSC NutriStem® XF Supplement Mix should be thawed at room temperature or at 2-8°C. Avoid repeated freeze-thaw cycles (up to two times).

For a complete medium, aseptically add 0.6ml of MSC NutriStem® XF Supplement Mix to 100ml of MSC NutriStem® XF Basal Medium.

[Alternatively, add 3ml of MSC NutriStem® XF Supplement Mix to 500ml of MSC NutriStem® XF Basal Medium].

MSC NutriStem® XF Basal Medium contains L-glutamine.

Store at 2-8°C. Protect from light.

The complete MSC NutriStem® XF Medium is stable at 2-8°C for up to 30 days.

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⁺⁺, Catalog 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 CO₂ incubator (37°C).

4. Following 30 minutes incubation:

For immediate use:

- Gently wash the cultureware with DPBS (For T-25 use at least 5ml).
- 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 procedure

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

Culturing of hMSC in the complete MSC NutriStem® XF Medium

1. Recovery of Cryopreserved hMSC

- 1.1 Pre warm 5-10 ml of complete MSC NutriStem® XF Medium in a 50 ml conical tube.
- 1.2 Rapidly thaw frozen vial of hMSC in a 37°C water bath, with agitation until a small amount of ice remains.
- 1.3 Slowly add (drop by drop while gently swirling) the cells into the pre-warmed complete MSC NutriStem® XF Medium.
- 1.4 Centrifuge cells at 300-400xg for 4-5 minutes at room temperature.
- 1.5 Remove supernatant and re-suspend cell pellet in 0.5-1 ml of complete MSC NutriStem® XF Medium.
- 1.6 Perform a viable cell count (e.g., using Trypan Blue Exclusion Assay)
- 1.7 Add the desired volume of complete MSC NutriStem® XF Medium.
- 1.8 Transfer the cells into MSC Attachment Solution pre-coated cultureware (see above). Seeding densities should be calculated (see table 2).
- 1.9 Incubate in a humidified CO₂ incubator (37°C).

Note:

It is possible to avoid the centrifugation step after thawing. In this case skip steps 1.4-1.5 and transfer the thawed cells (from Step 1.3) 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).

2. 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 4500-5500 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 3-4 days post seeding). avoid overgrown culture, as it leads to cell's maturation and senescence

Subculturing Protocol Overgrowth culture will enhance cells maturation

- 2.1 Remove culture medium and gently wash once with DPBS w/o Ca, Mg (Cat. No. 02-023-1).
- 2.2 For T25 culture flask add 1-3ml 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.
- 2.3 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.
- 2.4 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
- 2.5 Centrifuge cells for 4-5 minutes at 300-400xg at room temperature. Carefully discard the supernatant.
- 2.6 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 3
- 2.7 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).
- 2.8 Incubate in a humidified CO₂ incubator (37°C).
- 2.9 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 cm ²	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 x 10 ⁴ cells / well	4.3-5.3 x 10 ⁴ cells / well	11-14 x 10 ⁴ cells / T-25

3. Cryopreservation of hMSC

- 3.1 Rapidly re-suspend hMSC pellet with cold MSC Freezing Solution (Cat. No. 05-712-1) (recommended between 0.5-1x10⁶ cells/ml, 1ml/vial).
- 3.2 Immediately place the cryovials in appropriate freezing container (e.g., "Mr. Frosty") and place at -80°C for overnight.
- 3.3 Transfer the cryovials into liquid nitrogen.

Quality Control

MSC NutriStem® XF Medium performance is tested for optimal maintenance and expansion of undifferentiated hMSC, while maintaining their multi-lineage differentiation potential. Additional tests are: pH, osmolality, endotoxins and sterility tests.

Auxiliary Products

Product Name	Cat. No.
MSC Attachment Solution	05-752-1
MSC Freezing Solution	05-712-1
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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