



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

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 and umbilical cord tissue; BM-hMSC, AT-hMSC, UCT-hMSC).

For the initial isolation of hMSC it is recommended to add 2.5% human AB serum to the complete medium to facilitate cell's attachment and growth.

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

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. The pre-coated step with MSC Attachment Solution is also required for the initial isolation of hMSC using human AB serum.

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

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 5000-6000 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.

Subculturing Protocol

- 2.1 Pre-warm Recombinant Trypsin Solution (with or without EDTA, cat. no. 03-078-1, 03-079-1) to room temperature before use.
- 2.2 Remove culture medium and gently wash once with DPBS w/o Ca, Mg (Cat. No. 02-023-1).
- 2.3 For T25 culture flask add 1-3ml of Recombinant Trypsin Solution. (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.
- 2.4 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.
- 2.5 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
- 2.6 Centrifuge cells for 4-5 minutes at 300-400xg at room temperature. Carefully discard the supernatant.
- 2.7 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
- 2.8 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).
- 2.9 Incubate in a humidified CO₂ incubator (37°C).
- 2.10 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	1.8-2.3 x 10 ⁴ cells / well	4.5-5.5 x 10 ⁴ cells / well	12-15 x 10 ⁴ cells / T-25

3. Cryopreservation of hMSC

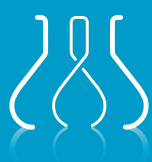
- 3.1 Rapidly re-suspend hMSC pellet with cold MSC Freezing Solution (Cat. No. 05-712-1) (recommended between 0.5-1x10⁶ cells/ml, 1ml/vial).
- 3.2 Immediately place the cryovials in appropriate freezing container (e.g., "Mr. Frosty") and place at -80°C for overnight.
- 3.3 Transfer the cryovials into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