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MSC NutriStem® XF Medium

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

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's 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 hMSCs 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's using human AB serum.
For optimal cell passage and long term culture of the cells, it is recommended to use the MSC Dissociation Solution (Cat. No. 03-075-1) or 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 hMSCs from different sources.
- Supports long-term growth of hMSCs, 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 hMSCs to MSC NutriStem® XF Medium

hMSCs 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-glutmine.
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 Culture Dishes with MSC Attachment Solution (Cat. No. 05-752-1)

1. Dilute the MSC Attachment Solution 1:100 using sterile DPBS (without Ca++ and Mg++, Cat. No. 02-023-1) and gently mix using a pipette.
2. Coat the desired culture dish with the 1:100 diluted MSC Attachment Solution.
   The volume should be adequate for covering the desired well or plate.
   Use Table 1 for the recommended volumes.
3. Gently agitate the coated dish. Verify complete covering of the surface dish.
4. Incubate for at least 30 minutes in a humidified CO_2 incubator (37°C).
   Alternatively, wrap the coated dishes with Parafilm® and incubate at 2-8°C overnight, to avoid drying.
5. Before seeding, gently wash the culture vessel with DPBS.

Table 1. Recommended volume for the coating procedure

Culture vessel	Surface area cm^2	Volume of 1:100 MSC Attachment Solution
96-well plate	0.3	0.1 ml
24-well plate	1.9	0.4 ml
12-well plate	3.9	0.8 ml
6-well plate	9.6	2 ml
T25 Flask	25	5 ml
T75 Flask	75	15 ml

Notes:
1. Longer incubation (up to 72 hours) of culture vessel in a humidified CO_2 incubator (37°C) with MSC Attachment Solution will not decrease cell attachment and spreading performance.
2. Coated culture dishes stored under sterile conditions at +2-8°C should be used within 1 week.
   Culture dish should be wrapped with parafilm to avoid drying.

Culturing of hMSCs in the complete MSC NutriStem® XF Medium

1. Recovery of Cryopreserved hMSCs

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 hMSCs in a 37°C water bath, with agitation untill 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 culture dish (see above). Seeding densities should be calculated (see table 2).

1.9 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 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 hMSCs

MSC NutriStem® XF Medium was developed for optimal proliferation of hMSCs 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 hMSCs in MSC NutriStem® XF Medium, it is recommended to seed hMSCs at a concentration of 5000-6000 cell/cm^2 (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.
Note: The following sub-culture protocol is based on using MSC Dissociation Solution (Cat. No. 03-075-1). Alternatively, it is possible to use other hMSC qualified dissociation solutions, such as Non-Enzymatic MSC Dissociation Solution (Cat. No. 03-077-1) or Recombinant Trypsin Solutions (Cat. No. 03-078-1, 03-079-1). In such cases, use the corresponding product instruction manual for the dissociation procedure.

Subculturing Protocol

2.1 Pre-warm the MSC Dissociation Solution (Cat. No. 03-075-1) 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 MSC Dissociation Solution. (For any other culture dish, the appropriate volume should be adjusted).
Note: The more the culture is confluence and/or highly passaged, 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 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, suspend the cells in 5-10 ml of DPBS or pre-warmed MSC NutriStem® XF Medium. Collect cell suspension into sterile tube and re-wash the culture dish 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.

2.8 Re-seed cells into pre-coated culture dish (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 CO_2 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/cm^2)

Culture vessel	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 hMSCs

3.1 Rapidly re-suspend hMSC pellet with cold MSC Freezing Solution (Cat. No. 05-712-1) (recommended between 0.5-1x10^6 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 hMSCs, 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 Dissociation Solution	03-075-1
MSC Freezing Solution	05-712-1
Non-Enzymatic MSC Dissociation Solution	03-077-1
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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Culture of Excellence

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