

# PromoExQ MSC Growth Medium XF

## Instruction manual

Product	Size	Catalog number
PromoExQ MSC Growth Medium XF – includes Basal Medium and SupplementMix	500 ml	EQ-C-28019

### Recommended for

- Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM)
- Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC)
- Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT)

### Product description

Our PromoExQ MSC Growth Medium XF is a serum-free and xeno-free culture medium developed for the GMP compliant<sup>1</sup> *in vitro* expansion of multipotent mesenchymal stem cells (MSC) from bone marrow, the umbilical cord matrix (Wharton's Jelly), and adipose tissue. The PromoExQ MSC Growth Medium XF consists of a bottle of Basal Medium and one vial of SupplementMix. Adding the SupplementMix to the Basal Medium results in the complete Growth Medium.

### Supplementation details

Our PromoExQ MSC Growth Medium XF contains all the growth factors and supplements necessary for the optimal expansion of human mesenchymal stem cells.

**Note:** The PromoExQ MSC Growth Medium XF does not contain antibiotics or antimycotics and is formulated for use in an incubator with an atmosphere of 5% CO<sub>2</sub>.

### Culture vessel surface coating

Our PromoExQ MSC Growth Medium XF does not contain attachment- and spreading factors. Therefore, culture vessels to be used with Mesenchymal Stem Cell Growth Medium XF must be precoated either with 1 µg/cm<sup>2</sup> human fibronectin or 0.5 µg/cm<sup>2</sup> human vitronectin according to the instruction manual of the manufacturer.

### Preparation of the supplemented medium for use

Thaw the SupplementMix in a 37°C water bath with occasional swirling. Do not incubate longer than necessary. In case of visible precipitates after complete thawing, mix gently until all precipitates have redissolved. Then, transfer the entire content of the SupplementMix to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

### MSC subculture

For routine subculture of hMSC the use of Accutase is recommended with all MSC Growth Media.

### Storage and stability

The Basal Medium is shipped at room temperature and should be stored at 4 to 8°C in the dark upon arrival. The SupplementMix

is shipped on dry ice and should be stored at -20 to -16°C immediately after arrival. Do not freeze the Basal Medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the supplements to the Basal Medium, the complete PromoExQ MSC Growth Medium XF is stable for 3 weeks at 4 to 8°C. Do not freeze the complete medium.

For use, pre-warm only an aliquot of the complete medium at 15 to 25°C and keep the remaining medium refrigerated at 4 to 8°C.

### Quality control

All lots of PromoCell PromoExQ MSC Growth Medium XF are subjected to comprehensive quality control tests using primary human mesenchymal stem cells. Each lot of PromoExQ MSC Growth Medium XF is checked for growth promoting activity and for the absence of microbial contaminants. For details see lot specific Certificate of Analysis.

### Intended use

The regulated products are intended for research use or further manufacturing. They are not intended for direct administration to humans or animals. For safety precautions please see appropriate MSDS.

<sup>1</sup> according to international guidelines

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# Thawing and expansion of cryopreserved mesenchymal stem cells

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## Thawing protocol

### Materials

- PromoExQ MSC Growth Medium XF (EQ-C-28019)
- Human MSCs from bone marrow (hMSC-BM), umbilical cord matrix (hMSC-UC), or adipose tissue (hMSC-AT)
- Human fibronectin or vitronectin solution

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### Prepare the medium and the culture vessel

Calculate the required culture surface area according to the plating density and the lot-specific cell numbers stated on the certificate of analysis. The recommended plating density for our MSCs is 4,000 cells per cm<sup>2</sup>. Coat the culture vessel either with 1 µg/cm<sup>2</sup> fibronectin or 0.5 µg/cm<sup>2</sup> vitronectin according to the instruction manual of the manufacturer. Fill the appropriate volume of PromoExQ MSC Growth Medium XF (at least 9 ml per vial of cells) in the fibronectin- or vitronectin-coated cell culture vessels. Place the vessels in an incubator (37°C, 5% CO<sub>2</sub>) for 30 minutes.

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### Thaw the cells

Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for two minutes. Ensure that no water enters the thread of the screw cap.

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### Disinfect the vial and seed the cells

Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a cell culture vessel containing the prewarmed medium from step 1.

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### Incubate the cells

Place the vessel in an incubator (37°C, 5% CO<sub>2</sub>) for cell attachment. Replace the PromoExQ MSC Growth Medium XF after three to four hours to avoid damaging of the cells by DMSO. Change the medium every two to three days thereafter. The cells should be subcultured according to the subcultivation protocol once they have reached 70-90% confluency.

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## Subcultivation protocol

### Materials

- PromoExQ MSC Growth Medium XF (EQ-C-28019)
- Accutase-Solution
- Hepes BSS Solution

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#### Prepare the reagents and wash the cells

Place the Accutase-Solution at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the PromoExQ MSC Growth Medium XF from the culture vessel. Add 100 µl Hepes BSS Solution per cm<sup>2</sup> of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.

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#### Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Accutase-Solution per cm<sup>2</sup> of vessel surface and incubate for two to four minutes at room temperature. Close the vessel and examine the cells under a microscope.

When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.

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#### Harvest the cells

Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for three minutes at 220 x g.

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#### Incubate the cells

Discard the supernatant (step 1), add 1 ml of PromoExQ MSC Growth Medium XF, and resuspend the cells by carefully pipetting up and down.

Plate the cells with 4,000 cells per cm<sup>2</sup> in new cell culture vessels containing prewarmed PromoExQ MSC Growth Medium XF. Place the vessels in an incubator (37°C, 5% CO<sub>2</sub>) and change the media every two to three days.

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If you require special media modifications, we offer a GMP compliant media production service.  
Contact us at [info@promocell.com](mailto:info@promocell.com) to find out more.

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