

# Mesenchymal Stem Cell Media

PromoCell

## Instruction Manual

### ■ Mesenchymal Stem Cell Growth Medium

Product	Size	Catalog Number
Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use)	500 ml	C-28009
Mesenchymal Stem Cell Growth Medium DXF (Ready-to-use)	500 ml	C-28019

### ■ Mesenchymal Stem Cell Differentiation Medium

Product	Size	Catalog Number
Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (Ready-to-use)	100 ml	C-28016
Mesenchymal Stem Cell Chondrogenic Differentiation Medium (Ready-to-use)	100 ml	C-28012
Mesenchymal Stem Cell Osteogenic Differentiation Medium (Ready-to-use)	100 ml	C-28013
Mesenchymal Stem Cell Chondrogenic Differentiation Medium w/o Inducers (Ready-to-use)	100 ml	C-28014
Mesenchymal Stem Cell Neurogenic Differentiation Medium (Ready-to-use)	100 ml	C-28015

#### 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

PromoCell Mesenchymal Stem Cell Media were developed for the *in vitro* expansion and directed differentiation of mesenchymal stem cells (MSC) from bone marrow, the umbilical cord matrix (Wharton's Jelly) and adipose tissue. PromoCell Mesenchymal Stem Cell Media are available as Medium (Ready-to-use) and consist of a bottle of Basal Medium and one vial of Supplement-Mix. Adding the Supplement-Mix to the

Basal Medium results in the complete Medium.

The PromoCell Mesenchymal Stem Cell Growth Medium 2 (C-28009) is an optimized low-serum formulation for routine culture of human MSC. The Mesenchymal Stem Cell Growth Medium DXF (C-28019) is a defined and xeno-free culture medium and requires fibronectin-coated vessels (see below).

#### Supplementation Details

PromoCell Mesenchymal Stem Cell Media contain all the growth factors and supplements necessary for the optimal expansion and directed differentiation of human mesenchymal stem cells.

**Note:** The PromoCell MSC Chondrogenic Differentiation Medium w/o Inducers (C-28014) must be supplemented with adequate chondrogenic inducers by the customer.

The PromoCell MSC Growth Medium DXF, the MSC Chondrogenic Differentiation Medium (C-28012), the MSC Chondrogenic Differentiation Medium w/o Inducers (C-28014) and the MSC Neurogenic Differentiation Medium (C-28015) are defined and serum-free.

PromoCell Mesenchymal Stem Cell Media do not contain antibiotics or antimycotics and are formulated for use in an incubator with an atmosphere of 5% CO<sub>2</sub>.

#### Culture vessel surface coating

PromoCell MSC Growth Medium DXF does not contain attachment- and spreading factors. Therefore, culture vessels to be used with MSC Growth Medium DXF must be precoated with 10 µg/ml human Fibronectin (C-43060) according to the instruction manual.

Alternatively, bovine Fibronectin (C-43050) may be used.

### 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 re-dissolved.** Then, transfer the entire content of the Supplement-Mix 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 (C-41310) is recommended with all PromoCell MSC Growth Media. The PromoCell Cell Dissociation Solution ACF (C-41320) is the detachment reagent of choice for a defined and xeno-free subculture process.

### Instructions for the Use of PromoCell MSC Differentiation Media

#### Adipogenic Differentiation Protocol

1. Coat a 6-well tissue culture plate with 10 µg/ml human or bovine fibronectin (C-43060/C-43050) according to the instruction manual.
2. In a fibronectin-coated 6-well tissue culture plate, plate 1 x 10<sup>5</sup> MSC per well using MSC Growth Medium 2 (C-28009). Work in duplicate.
3. Allow the cells to reach 80–90% confluency. This will take 24–48 hours.
4. Induce one of the duplicate samples with MSC Adipogenic Differentiation Medium 2 (C-28016). Use MSC Growth Medium 2 for the remaining well as a negative control.
5. Incubate for 12–14 days. Change the medium every third day taking care not to disturb the cell monolayer.

#### Chondrogenic Differentiation Protocol

1. The negative control medium is Dulbecco's Modified Eagle's Medium

(DMEM, low-glucose) with 2 mM L-glutamine and 10% fetal calf serum.

2. Plate MSC at 2 x 10<sup>5</sup> cells per well in a 96-well U-bottom suspension culture plate using the negative control medium. Work in duplicate.

**Note:** The more cells you use, the larger the spheroids. Up to 3 x 10<sup>5</sup> cells per well can be plated.

3. Spheroids will spontaneously form within 24–48 hours of incubation.
4. Induce one of the duplicate samples with MSC Chondrogenic Differentiation Medium (C-28012). Use the negative control medium for the remaining wells.
5. Incubate for 21 days. Change the medium every third day taking care not to aspirate the spheroids.

#### Osteogenic Differentiation Protocol

1. Coat a 6-well tissue culture plate with 10 µg/ml human or bovine fibronectin (C-43060/C-43050) according to the instruction manual.
2. Plate MSC at 1x10<sup>5</sup> cells per well in the fibronectin-coated tissue culture plate using MSC Growth Medium 2 (C-28009). Work in duplicate.
3. **Important:** Allow the cells reach at least 100% confluency. This will take 48–72 hours.
4. Induce one of the duplicate samples with MSC Osteogenic Differentiation Medium (C-28013). Use MSC Growth Medium 2 for the remaining well as a negative control.
5. Incubate for 12–14 days. Change the medium every third day. Be careful not to disturb the cell monolayer.

#### Neurogenic Differentiation Protocol

1. Coat a 6-well tissue culture plate with 10 µg/ml human or bovine fibronectin (C-43060/C-43050) according to the instruction manual.
2. Plate 4 x 10<sup>3</sup> cells/cm<sup>2</sup> on the fibronectin-coated plate using MSC Growth Medium 2 (C-28009). Work in duplicate. Culture the cells to 60–80% confluency. Change the medium every 48 hours.
4. Induce one of the duplicate samples with MSC Neurogenic Differentiation Medium (C-28015). Use MSC Growth Medium 2 for the remaining well as a negative control.

5. Incubate for at least 3 days. Change the medium every 48 hours.

**Note:** Significant morphological changes in the cells can be observed as early as 1 day after induction.

For detailed information, please see [www.promocell.com/application-notes](http://www.promocell.com/application-notes).

### Storage and Stability

Store the Basal Medium at 4–8°C in the dark and the SupplementMix at -20°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 shelf life of the complete medium is 6 weeks at 4–8°C. The complete MSC Growth Medium DXF is stable for 3 weeks at 4–8°C. Do not freeze the complete medium.

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

**Note:** The SupplementMix is delivered thawed and can be frozen after arrival without losing any activity.

### Quality Control

All lots of PromoCell Mesenchymal Stem Cell Media are subjected to comprehensive quality control tests using primary human mesenchymal stem cells. Each lot of MSC Growth Medium is checked for maintenance of multipotency, growth promoting activity, adherence rate, and typical morphology of the tested mesenchymal stem cells. Each lot of MSC Differentiation Media is tested for the capacity to induce directed differentiation into the respective lineages in MSC. In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria, mycoplasma).

### Intended Use

The products are for *in vitro* use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

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