Mesenchymal stem cell media

PromoCell®

Instruction manual

Mesenchymal Stem Cell Growth Media

Product	Size	Catalog number
Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use)	500 ml	C-28009
Mesenchymal Stem Cell Growth Medium 2, phenol red-free (Ready-to-use)	500 ml	C-28017

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 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

Our 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.

The Mesenchymal Stem Cell Media are available as medium (Ready-to-use) and consist of a bottle of basal medium and one vial of SupplementMix. Adding the SupplementMix to the basal medium results in the complete medium.

The Mesenchymal Stem Cell Growth Medium 2 (C-28009) and Mesenchymal Stem Cell Growth Medium 2, phenol red-free (C-28017) are optimized low-serum formulation for routine culture of human MSC. The MSC Chondrogenic Differentiation Medium (C-28012) and the MSC Neurogenic Differentiation Medium (C- 28015) are serum-free.

Supplementation details

Our 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 Mesenchymal Stem Cell Media do not contain antibiotics or antimycotics and are formulated for use in an incubator with an atmosphere of 5% CO_2.

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 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 (C-41310) is recommended with all MSC Growth Media.

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 the supplements are added to the basal medium, store at 4–8°C and use within 6 weeks. For use, pre-warm only an aliquot of the complete medium at 15–25°C and keep the remaining medium refrigerated at 4–8°C.

Quality and 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 Mesenchymal Stem Cell Growth Media is checked for growth promoting activity of our mesenchymal stem cells tested for multipotency as proposed by the ISCT [1]. Each lot of MSC Differentiation Media is tested for the capacity to induce directed differentiation into the respective lineages in MSCs expanded in our Mesenchymal Stem Cell Growth Medium 2. In addition, all lots of media have been tested for the absence of microbial contaminants (fungi and bacteria).

For details see lot specific Certificate of Analysis.

Intended use

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

For detailed information, please refer to our application note on our website www.promocell.com

Instructions for the use of MSC differentiation media

I. Adipogenic differentiation

1

Coat a 6-well tissue culture plate with 0.5 µg/cm² human vitronectin according to the instruction manual.

Note: 1 µg/cm² human fibronectin may also be used for coating.

2

In a vitronectin-coated 6-well tissue culture plate, plate 1 × 10^5 MSC per well using MSC Growth Medium 2 (C-28009). Work in duplicate. Allow the cells to reach 80–90% confluency. This will take 24- 48 hours.

3

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.

4

Incubate for 12–14 days. Change the medium every third day taking care not to disturb the cell monolayer.

II. Chondrogenic differentiation

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 × 10^5 cells per well in a 96-well U-bottom suspension culture plate using the negative control medium. Work in duplicate. Spheroids will spontaneously form within 24–72 hours of incubation.

Note: The more cells you use, the larger the spheroids. Up to 3 × 10^5 cells per well can be plated.

3

Induce one of the duplicate samples with 200 µl of MSC Chondrogenic Differentiation Medium (C-28012). Use the negative control medium for the remaining wells.

4

Incubate for 21 days. Change the medium every 2–3 days taking care not to aspirate the spheroids.

III. Osteogenic differentiation

1

Use collagen I precoated 6-well tissue culture plates (e.g., Gibco™) and work with 3 ml MSC Growth Medium 2 (C-28009) per well.

2

Plate MSC with a seeding density of 5,000 cells /cm² in each well of the collagen I precoated plate, e.g. 48,000 cells per well, using MSC Growth Medium 2 (C-28009). Work in duplicate. Change medium every 2–3 days and use 3 ml medium per well for a 6-well plate.

Important: Allow the cells reach at least 80–90% confluency. This will take 4–6 days.

3

Induce one of the duplicate samples with 3 ml MSC Osteogenic Differentiation Medium (C-28013). Use 3 ml MSC Growth Medium 2 for the remaining well as a negative control.

4

Incubate for 15 days. Change the medium every 2–3 days (e.g. Monday, Wednesday, Friday). Be careful not to disturb the cell monolayer.

IV. Neurogenic differentiation

1

Coat a 6-well tissue culture plate with 0.5 µg/cm² human vitronectin according to the instruction manual.

Note: 1 µg/cm² human fibronectin may also be used for coating.

2

Plate 10,000 cells/cm² on the vitronectin-coated plate using MSC Growth Medium 2 (C-28009). Work in duplicate. Culture the cells to 50–90% confluency. Change the medium every 2–3 days.

3

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.

4

Incubate for at least 4 days. Change the medium every every 2–3 days.

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

References

[1] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, et al., Cytother 2006, 8(4):315–7.

If you require special media modifications, we offer a custom media service starting at 10 bottles per order.
Contact us at info@promocell.com to find out more.

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