Datasheet

PowerStem MSC1

Xeno- and Serum-free Medium for the Cultivation and Proliferation of Human Mesenchymal Stem Cells (hMSC)

Product	Description	Catalogue-No.	Size
PowerStem MSC1	Xeno-free and serum-free medium for cultivation and expansion of mesenchymal stem cells, Kit (Basal Medium + 2 Supplements) for 500 ml	P04-77355K	500 ml

Product description

PowerStem MSC1 is an easy to use xeno- and serum-free medium for cultivation and proliferation of human mesenchymal stem cells (hMSC). PowerStem MSC1 is especially designed for the proliferation of human mesenchymal stem cells without differentiation. PowerStem MSC1 supports long-term growth of MSC and preserves their multi-lineage potential. In addition, MSC cultured in PowerStem MSC1 expand faster and show a significant reduction in hematopoietic cell contamination at early passages compared to serum-based media. To differentiate the proliferated MSCs into different cell types the relevant protocols and differentiation factors should be used.

The product is manufactured under the highest standards and quality is checked continuously and strictly. PowerStem MSC1 is made from selected raw materials of the highest quality. The medium contains salts, amino acids, trace elements, hormones, growth factors and enriched human proteins derived from human blood in an optimized formulation. Because of this, human blood components precipitates may occur in rare cases despite the addition of heparin after prolonged storage. These precipitates consist in most cases of fibrin. Fibrin appears in medium as larger material (up to 1-2mm) that is visible to the naked eye. The precipitate is often mistaken for microbial contamination. The quality and suitability of the medium in cell culture is thereby in no way limited.

Content

PowerStem MSC1 medium consists of:

- PowerStem MSC1 basal medium (470 ml, Cat. No. P04-77350C)
- PowerStem MSC1 growth supplement 1 (5 ml, Cat. No. P04-77350S1) which is added at the time of use.
- PowerStem MSC1 growth supplement 2 (25 ml, Cat. No. P04-77350S2) which is added at the time of use.

Storage conditions and stability:

- PowerStem MSC1 basal medium: store in the dark at 2-8° C
- Two PowerStem MSC1 growth supplements: store in the dark at -20° C (will be shipped on dry ice, should be used immediately on arrival or may be refrozen for later use)

PowerStem MSC1 basal medium is stable for 2 years and PowerStem MSC1 growth supplements are guaranteed stable for 12 months when properly stored. PowerStem MSC1 complete medium (basal + supplements) is stable for 6 weeks when stored in the dark at 2-8° C. We do not recommend using the supplemented complete medium beyond 6 weeks. Do not freeze complete PowerStem MSC1 medium.

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Composition

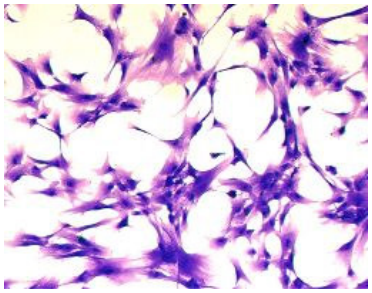
PowerStem MSC1 contains salts, amino acids, trace elements, hormones, growth factors, and enriched human proteins and lipids in an optimized formulation. PowerStem MSC1 is free of (non-human) animal derived components.

Suitability

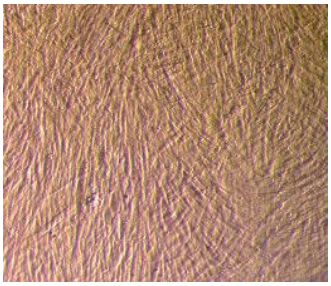
Serum-free cultivation of human mesenchymal stem cells (hMSC) while maintaining the undifferentiated state and multi-lineage potential. Please note: For research use only, not for therapeutic or diagnostic use.

Special Advantages

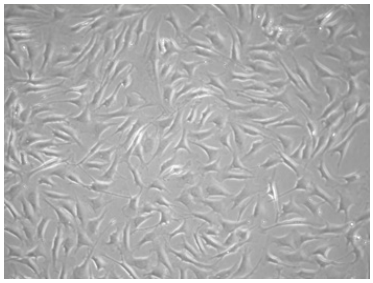
PowerStem MSC1 allows the cultivation of human mesenchymal stem cells under serum-free conditions. It is free of animal or human serum and thus enables constant and comparable experimental conditions resulting in highly reproducible data. PowerStem MSC1 is completely free of animal components (ADCF) and thus suitable for a research approach in regenerative medicine and tissue engineering. By adding specific differentiation factors, MSC can be differentiated in vitro to the desired cell types (bone, cartilage, adipose tissue etc.).



Sub-confluent hMSC in PowerStem MSC1



Confluent hMSC in PowerStem MSC1



hMSC in medium with 10% FBS

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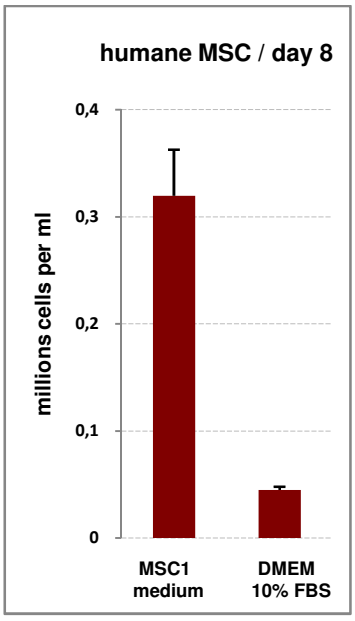
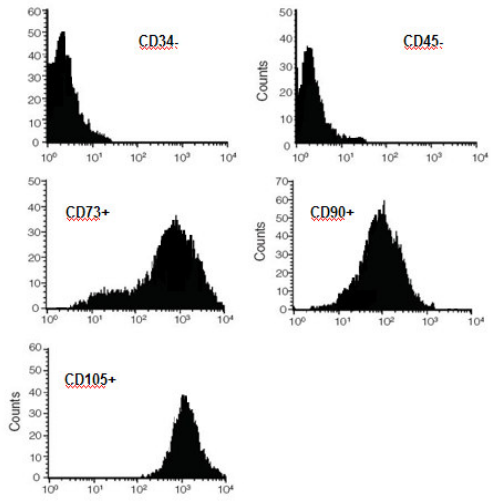
humane MSC proliferation

Picture is showing the average of three independent experiments. Growth conditions: 2000 cells per ml / growth area 1.9 cm^2.

During the growth phase medium was not changed.

After 8 days the cell density was determined





The culture-expanded cell population expresses CD90 (Thy-1), CD105 (SH2) and CD73 (SH3/SH4), but lacks expression of CD34 and CD45.

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Preparation of PowerStem MSC1 medium

PowerStem MSC1 basal medium requires supplementation with PowerStem MSC1 growth supplements. Thaw PowerStem MSC1 growth supplements before use. The thawed growth supplements should be added to the basal medium immediately. PowerStem MSC1 complete medium (basal medium with growth supplements) is stable for 6 weeks when stored in the dark at 2-8° C.
Antibiotics can be used if desired, we recommend Gentamicin Reagent Solution (5 µg/mL final concentration).

Culture Conditions

Media: Complete PowerStem MSC1 medium
Cell- Line(s): Human mesenchymal stem cells (hMSC), adipose-derived stem cells (ADSC) or ASC/TERT1.
Culture Type: Adherent
Culture Vessels: CELLstart™ CTS™-coated T-Flasks.
Temperature Range: 36°C to 37.8°C
Incubator Atmosphere: Humidified atmosphere of 4–6% CO_2 in air.
Ensure proper gas exchange and minimize exposure of cultures to light.
Note: Procedures detailed in the following sections are for cultures in T-75 culture flasks (75 cm^2).
Volumes should be adjusted accordingly for desired vessel size.

Recovery

- Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
- Pipette the entire content of the cryovial in a sterile 50-ml conical tube.
- Ad carefully 5–10 ml of pre-warmed (37°C) complete PowerStem MSC1 medium.
- Ensure homogeneity of the cell suspension by regular gentle blending the tube.
- Centrifuge cell suspension at 100–200 × g for 5 minutes at room temperature.
- Aspirate and discard supernatant being careful not to disturb the cell pellet.
- Resuspend the cell pellet in a minimal volume of pre-warmed complete PowerStem MSC1 medium for cell counting. Determine total viable cell density. Calculate the volume of cell suspension required to seed cells at a density of approximately 5 × 10^3 cells/cm^2.
- Add cell suspension to an appropriate PowerStem MSC1 coated T-75 flask containing 10–15 ml pre-warmed complete PowerStem MSC1 medium.
- Incubate at 37˚C in a humidified atmosphere of 5% CO_2 in air.
- Replace the medium in the flasks every 3–4 day.

Coating Culture Flasks with Fibronectin-Solution

Coating of cell culture vessels using Fibronectin - attachment factor (2705001S PAN-Biotech).

- Fibronectin working concentration 1-5µg/cm^2
- Coat the bottom of cell culture flask with solution
- ensure complete surface coverage
- incubate for 2 hours at 37° C
- Aspirate remaining solution
- Let dry for 30 minutes under tissue hood.

Optimal conditions for attachment must be determined for each cell line and application.

Subculture in PowerStem MSC1

- Observe stock culture with microscope and confirm that the cells are ready to be subcultured (60–80% confluent).
- Aspirate spent medium and wash 2x with 5 ml of DPBS (without Mg^{2+}/Ca^{2+}) per T25 flask; adjust volume according to size of culture vessel (e.g. 10 ml per T75, 1 ml per 24-well etc.).
- Add Accutase (P10-21100 Pan-Biotech) to culture vessel (5 ml per T25) in aseptic procedure.

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- Dispense enough Accutase solution into culture vessel(s) to completely cover the monolayer of cells 1-2 minutes than remove excess Accutase solution.
- Cells detach at room temperature within 5 to 10 min.
- Tap flask to release cells.
- Resuspend cells in fresh cell culture medium and determine cell amount by trypan blue exclusion staining.
- Split cells to desired cell density and transfer to a new culture vessel.
- Incubate at 37°C in a humidified CO2 incubator.

NOTE: For embryonic stem cell cultures and cells cultured under serum-free conditions, a washing step is recommended to remove excess Accutase from cells; spin down cells as usual (e.g. 5 min at 200-300 g) remove Accutase and resuspend in fresh medium.

Freezing cells with Cryopan I

(P07-92500, P07-92050, P07-92100 PAN-Biotech)

For optimal results only vital cells in the log-growth phase should be used.

- Thaw Cryopan I and store till using at 2-8°C.
- Trypsinate adherent cells, transfer the cells into the culture medium, stop the trypsin activity with trypsin inhibitor and centrifuge.
- Discard the supernatant and wash the cell pellet in PBS (without Ca^{2+}/Mg^{2+}).
- After an additional centrifugation step (100 – 200 g, 5 - 10 minutes) transfer the cells into PBS and determine cell number and cell vitality by trypan blue cell viability staining.
- Centrifuge the cells again and discard the supernatant.
- Transfer the cells into the cold Cryopan I ($5x10^5$ - $2x10^6$ cells/ml Cryopan I).
- Suspend the cells carefully mixing the suspension by repeated pipetting until there are no more cell clumps.
- Refill the cell suspension into labelled cryotubes (0.5 – 1.5ml/tube).
- Freeze the cells in an automatically or manually controlled cryo freezer.
  The optimal freezing rate is approximately 1°C / minute.
- Alternatively, put the tubes for 15 minutes into a refrigerator, so that the freezing medium can penetrate into the cells. After this step freeze the tubes at -20°C for 2 hours box and put them into the vapour phases of liquid nitrogen over night.
- After 24 hours transfer frozen cells to liquid nitrogen, (vapor phase) storage
  at –200°C to –125°C is recommended.

Technical support

For technical support, questions or remarks please contact your local PAN-Biotech partner or the technical department of PAN-Biotech via email (info@pan-biotech.com) or phone +49-8543-601630.
