

## Poietics™ human mesenchymal stem cells

### Instructions for use

---

#### Safety statements

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use *in vitro* diagnostic or clinical procedures.

**WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS**

**POTENTIALLY INFECTIOUS.** Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5<sup>th</sup> edition. If you require further information, please contact your site safety officer or scientific support.

#### Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact customer service.
3. BulletKit™ instructions: upon arrival, store basal medium at 4°C to 8°C and SingleQuotes™ at –20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4°C and added to basal medium within 72 hours of receipt. After SingleQuotes™ are added to basal medium, use within 1 month. Do not re-freeze.  
Using media or reagents other than what's recommended will void the cell warranty. Please contact scientific support if you need help selecting media and/or reagents.
4. Trypsin/EDTA solution has a limited shelf life or activation at 4°C. If, upon arrival, trypsin/EDTA is thawed, immediately aliquot and refreeze at –20°C.

**NOTE:** To keep trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and re-freeze at –20°C.

#### Preparation of media

1. Decontaminate the external surfaces of the MSCGM™ SingleQuotes™ cryovials and the mesenchymal stem cell basal medium (MSCBM) bottle with 70% v/v ethanol or isopropanol.
2. Aseptically open the bottle of mesenchymal cell growth supplement (MCGS). Add the contents to the 440 ml MSCBM.
3. Aseptically open each cryovial of L-glutamine and GA-1000 and add the entire amount from each SingleQuotes™ to the MSCBM.
4. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses should not affect the cell characteristics.
5. Use supplemented medium for the maintenance of hMSC only.

#### Thawing of cells / initiation of culture process

Please note we are in the process of changing the fill volume for the hMSC from 0.5 ml to 1 ml. Please check your vial for fill volume.

1. The recommended seeding density for human mesenchymal stem cells is 5,000-6,000 cells per cm<sup>2</sup>.
2. To set up cultures calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used. Add the appropriate amount of medium to the vessels (0.2-0.4 ml per cm<sup>2</sup>) and allow the vessels to equilibrate in a 37°C, 5% CO<sub>2</sub> humidified incubator for at least 30 minutes.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten. Quickly thaw the cryovial in a 37°C water bath.
4. Watch your cryovial closely; when the last sliver of ice melts remove it. Do not submerge it completely. Thawing the cells for longer than 1½ minutes results in less than optimal results.

5. Remove the cryovial immediately, wipe it dry, and transfer to a sterile field where the equilibrated flasks should be waiting, ready to seed. Rinse the cryovial with 70% alcohol, and then wipe to remove excess.
6. Using a micropipette, gently add the thawed cell suspension to 5 ml of temperature-equilibrated medium.
7. Centrifuge at 500 x g for 5 minutes at room temperature.
8. Resuspend the pellet in a minimum volume of temperature equilibrated mesenchymal stem cell growth medium (MSCGM™) by gently pipetting up and down. Count the total number of viable cells.
9. Add the calculated volume of cell suspension to each prepared flask and gently rock to disperse the cell suspension over the growth surface.
10. Incubate at 37°C, 5% CO<sub>2</sub> and 90% humidity.

## Subculturing

1. Aseptically remove and discard all of the spent media from the flasks.
2. Wash the attached cell layer with Dulbecco's phosphate buffered saline or an equivalent calcium and magnesium free balanced salt solution. Add the wash solution to the side of the flask opposite the attached cell layer. Rinse by rocking the flask back and forth several times. Aseptically remove and discard the wash solution.
3. Add a sufficient volume of Clonetics™ trypsin/EDTA (CC-3232) solution to cover the cell layer (approx. 0.05 ml/cm<sup>2</sup>). Gently rock the flask(s) to ensure that the cells are covered by the trypsin solution. Incubate at room temperature for 5 minutes, then observe under a microscope. If the cells are less than 90% detached, continue incubating and observe every 3 minutes. Tapping the flask or plate will expedite cell detachment. Do not incubate the cells longer than 15 minutes.
4. Once ≥90% of the cells are rounded and detached, stand the flasks on end for a minimal length of time to allow the cells to drain. Add an equal volume of temperature equilibrated MSCGM™ to each vessel. Disperse the solution by pipetting over the cell layer surface several times.
5. To remove the trypsin, centrifuge cells at approximately 600 x g for 5 minutes at room temperature.
6. Resuspend the cell pellet(s) in a minimal volume of temperature equilibrated MSCGM™ and remove a sample for counting.

7. Count the cells with a hemacytometer or cell counter and calculate the total number of cells. Make a note of your cell yield for later use.
8. If necessary, dilute the suspension with MSCGM™ to achieve the desired "cells/ml" and re-count the cells.
9. Assess cell viability using trypan blue.
10. Use the following equation to determine the total number of viable cells.

$$\text{Total \# of Viable Cells} = \frac{\text{Total cell count} \times \text{percent viability}}{100}$$

11. Determine the total number of flasks to inoculate by using the following equation. The number of flasks needed depends upon cell yield and seeding density.

$$\text{Total \# of Flasks to inoculate} = \frac{\text{Total \# of viable cells}}{\text{Growth area} \times \text{Rec. Seeding Density}}$$

12. Use the following equation to calculate the volume of cell suspension to seed into your flasks. Determine the volume of MSCGM™ to add to each flask so that the final culture volume is 0.2 – 0.4 ml per cm<sup>2</sup>.

$$\text{Seeding Volume} = \frac{\text{Total volume of diluted cell suspension}}{\text{\# of flasks as determined in step 11}}$$

13. Prepare flasks by labeling each flask with the passage number, strain number, cell type and date.
14. Add the appropriate volume of temperature equilibrated MSCGM™ as determined in step 12.
15. Incubate at 37°C, 5% CO<sub>2</sub> and 90% humidity.
16. 3 to 4 days after seeding, completely remove the medium. Replace with an equal volume of MSCGM™. Cultures will be near confluence by day 6 or 7 and ready to subculture.

## Maintenance

1. hMSC cultures should be fed 3-4 days after plating.
2. To feed the cultures, gently and completely remove the MSCGM™ from the culture vessel.
3. Replace with an equal volume of temperature equilibrated MSCGM™ and return the culture vessels to the incubator.
4. When seeded at 5,000-6,000 cells per cm<sup>2</sup> of surface area, hMSC should be near confluence by day 6 or 7. The hMSC are contact-inhibited and should be subcultured when they are just sub-confluent (approximately 90% confluent).

Cells are frozen in passage 2 and we recommend they are used by passage 5.

## Adipogenic assay procedure

### Prepare adipogenic induction medium

Adipogenic induction medium should be used once the hMSC have become 100% confluent (approximately 5-13 days). Prepare the medium before hMSC become confluent.

1. Decontaminate the external surfaces of the hMSC adipogenic induction medium (PT-3102B) and the following SingleQuots™ with 70% v/v ethanol or isopropanol:
  - a. h-insulin (recombinant)
  - b. L-glutamine
  - c. MCGS
  - d. dexamethasone
  - e. indomethacin
  - f. IBMX (3-isobutyl-1-methyl-xanthine)
  - g. GA-1000
2. Aseptically open the above SingleQuots™ and add the contents to the 170 ml of adipogenic induction medium.
3. Rinse each SingleQuots™ vial with the medium. It may not be possible to recover the entire contents of each SingleQuots™. Small losses should not affect the cell characteristics.
4. Use supplemented medium for the adipogenic induction of hMSC only. Store at 2°C to 8°C in the dark until needed.

### Prepare adipogenic maintenance medium

1. Decontaminate the external surfaces of the hMSC adipogenic maintenance medium (PT-3102A) and the following SingleQuots™ with 70% v/v ethanol or isopropanol:
  - a. h-insulin (recombinant)
  - b. L-glutamine
  - c. MCGS
  - d. GA-1000
2. Aseptically open the above SingleQuots™ and add the contents to the 170 ml of adipogenic maintenance medium.
3. Rinse each SingleQuots™ vial with the medium. It may not be possible to recover the entire contents of each SingleQuots™. Small losses should not affect the cell characteristics.
4. Store supplemented adipogenic maintenance medium at 2°C to 8°C in the dark until needed.

## Adipogenesis culture protocol

1. Plate  $2.1 \times 10^4$  hMSC per  $\text{cm}^2$  of tissue culture surface area in 0.2 to 0.3 ml of MSCGM™ per  $\text{cm}^2$  of tissue culture surface area.  
For example:  $2 \times 10^5$  cells in 2 ml medium per  $9.6 \text{ cm}^2$  well of a 6-well plate. Incubate the cells at 37°C, in a humidified atmosphere of 5%  $\text{CO}_2$ .
2. Feed the cells every 2-3 days by completely replacing the medium with fresh MSCGM™ until the cultures reach confluence (5-13 days). hMSC must be confluent, or post confluent, for optimal adipogenic differentiation.
3. At 100% confluence, 3 cycles of induction/maintenance will stimulate optimal adipogenic differentiation. Each cycle consists of feeding the hMSC with supplemented adipogenesis induction medium and culture for 3 days (37°C, 5%  $\text{CO}_2$ ) followed by 1-3 days of culture in supplemented adipogenic maintenance medium. Feed non-induced control hMSC with only supplemented adipogenic maintenance medium on the same schedule. Adipogenic cells are delicate and care should be used to avoid disrupting the numerous lipid vacuoles in the cells. Do not let the cells dry out when changing medium.
4. After 3 complete cycles of induction/maintenance, culture the hMSC for 7 more days in supplemented adipogenic maintenance medium, replacing the medium every 2-3 days.
5. The extent of adipogenic differentiation may be noted by microscopic observation of lipid vacuoles in the induced cells. To document the adipogenic differentiation, cultures may be assayed using AdipoRed™ assay reagent. Non-induced cells will have few, if any, lipid vacuoles.

## Chondrogenic assay procedure

### Prepare incomplete chondrogenic induction medium

1. Decontaminate the external surface of the differentiation basal medium – chondrogenic and the following SingleQuots™ with 70% v/v ethanol or isopropanol.
  - a. dexamethasone
  - b. ascorbate
  - c. ITS + supplement
  - d. GA-1000
  - e. sodium pyruvate
  - f. proline
  - g. L-glutamine
2. Aseptically open the above SingleQuots™ and add the contents to the 185 ml of differentiation

basal medium – chondrogenic to prepare the incomplete chondrogenic induction medium.

3. Rinse each SingleQuots™ vial with the medium. It may not be possible to recover the entire content of each SingleQuots™. Small losses should not affect cell characteristics.
4. Store the incomplete chondrogenic induction medium at 2°C to 8°C in the dark until needed.

### Prepare and aliquot TGF-β3

1. Resuspend the lyophilized TGF-β3 (Lonza PT-4124) with sterile 4mM HCl supplemented with 1 mg/ml BSA or HSA to a concentration of 20 µg/ml. For example, use 100 µl diluent for 2 µg of TGF-β3.

**NOTE:** Each µl of TGF-β3 will convert 2 ml of incomplete chondrogenic medium into complete medium.

2. Aliquot small volumes of TGF-β3 into freezer-safe tubes and store at less than –70°C for no more than 6 months. (For example, the TGF-β3 can be frozen in 5 µl aliquots. Each aliquot will be sufficient to supplement 10 ml of Incomplete chondrogenic induction medium.)

### Complete chondrogenic induction medium

1. After thawing, the aliquot of TGF-β3 may need to be centrifuged briefly at low speed to pull the small volume (e.g. 5 µl) to the bottom of the tube.
2. Pipette the volume of incomplete chondrogenic induction medium that you intend to supplement (e.g. 10 ml) into a tube.
3. To recover the full volume of TGF-β3, transfer 100 µl of this incomplete chondrogenic medium to the tube of TGF-β3.
4. Mix the solution by pipetting and transfer it back to the tube of chondrogenic induction medium. Repeat this process to be certain that you have recovered the TGF-β3. Cap and invert several times to mix.
5. The chondrogenic induction medium is now complete, and contains TGF-β3 at a final concentration of 10 ng/ml.

**NOTE:** Complete chondrogenic medium must be prepared fresh and used within 12 hours.

### Chondrogenesis culture protocol:

1. Calculate the total number of pellet cultures required for your experiment ( $2.5 \times 10^5$  hMSCs are needed to form each chondrogenic pellet). Transfer this amount of cells to an appropriate culture tube to wash the cells.
2. Wash the hMSCs with **incomplete** chondrogenic medium: Centrifuge the cells at

150 x g for 5 minutes at room temperature, and aspirate/ discard the supernatant. Resuspend the cells in 1 ml **incomplete** chondrogenic medium per  $7.5 \times 10^5$  cells, centrifuge again at 150 x g for 5 minutes and aspirate/discard the medium.

3. Resuspend the hMSCs in **complete** chondrogenic medium to a concentration of  $5.0 \times 10^5$  cells per ml.
4. Aliquot 0.5 ml ( $2.5 \times 10^5$  cells) of the cell suspension into 15 ml polypropylene culture tubes. Centrifuge the cells at 150 x g for 5 minutes at room temperature. DO NOT aspirate the supernatant or resuspend the pellet.

**NOTE:** Polypropylene tubes are used so that the cells do not adhere to the tube. Polystyrene tubes should not be used.

5. Loosen the caps of the tubes one half turn to allow gas exchange and incubate the tubes at 37°C, in a humidified atmosphere of 5% CO<sub>2</sub>. Do not disturb the pellets for 24 hours.
6. Feed the cell pellets every 2-3 days by completely replacing the medium in each tube (to avoid aspirating the pellets when aspirating the medium, attach a sterile 1-200 µl pipette tip to the end of the aspirating pipette). Add 0.5 ml of freshly prepared complete chondrogenic medium to each tube.
7. After replacing the medium, flick the bottom of each tube to ensure that the pellet is free-floating, loosen the caps and return the tubes to the 37°C incubator.
8. Chondrogenic pellets should be harvested after 14 to 28 days in culture. Pellets may be formalin fixed and paraffin embedded for histological processing or may be prepared for frozen sectioning. Thin sections may be slide-mounted and immunostained for type II collagen.

### Osteogenic assay procedure

#### Prepare osteogenic induction medium

1. Decontaminate the external surfaces of the hMSC differentiation basal medium – osteogenic and the following SingleQuots™ with 70% v/v ethanol or isopropanol:
  - a. dexamethasone
  - b. L-glutamine
  - c. ascorbate
  - d. penicillin/streptomycin
  - e. MCGS
  - f. β-glycerophosphate
2. Aseptically open the above SingleQuots™ and add the contents to the 170 ml of hMSC differentiation basal medium – osteogenic.

- Rinse each SingleQuots™ vial with the medium. It may not be possible to recover the entire contents of each SingleQuots™. Small losses should not affect the cell characteristics.
- Store the supplemented osteogenic differentiation medium at 2°C to 8°C in the dark until needed.

### Osteogenesis culture protocol:

- Plate  $3.1 \times 10^3$  hMSCs per  $\text{cm}^2$  of tissue culture surface area in 0.2-0.3 ml of MSCGM™ per  $\text{cm}^2$  tissue culture area. For example:  $3 \times 10^4$  cells in 2 ml medium per  $9.6 \text{ cm}^2$  well of a 6-well plate. Cells may peel during differentiation – wells may be coated with collagen to prevent this.
- Allow the cells to adhere to the culture surface for 4 to 24 hours in MSCGM™ at 37°C, in a humidified atmosphere of 5%  $\text{CO}_2$ .
- Induce osteogenesis by replacing the MSCGM™ with osteogenesis induction medium.
- Feed the induced hMSCs every 3-4 days for 2-3 weeks by completely replacing the medium with fresh osteogenesis induction medium. Feed non-induced control hMSCs with MSCGM™ on the same schedule.
- Osteogenic induced cells will show changes in cell morphology, from spindle shaped to cuboidal shaped, as they differentiate and mineralize. Gaps may form in the post confluent cell layer and cells may begin to delaminate from culture surface. If this de-lamination is observed, proceed immediately to analysis of osteogenic differentiation as indicated by calcium deposition, or use the induced cells for other assays requiring osteocytes.
- For calcium deposition assays, harvest cells by rinsing them in calcium free PBS, then scraping cells from the culture surface in the presence of 0.5M HCl. Assay the extracts from osteogenic induced cultures for calcium content and compare to extracts from non-induced control cells.

### Ordering information

PT-2501	hMSC - human mesenchymal stem cells	$\geq 750,000$ cells
---------	-------------------------------------	----------------------

### Related products

#### Mesenchymal stem cell growth medium

PT-3001	MSCGM™ BulletKit™	MSCBM (440 ml) plus SingleQuots™ of growth Supplements
---------	-------------------	--

PT-3238	MSCBM	Mesenchymal stem cell basal medium (440 ml)
PT-4105	MSCGM™ SingleQuots™	Formulates MSCBM to MSCGM™. Contains mesenchymal cell growth supplement (MCGS), L-glutamine, and GA-1000.

### Differentiation media BulletKits – adipogenic

PT-3004	hMSC differentiation BulletKit™ – adipogenic	Contains adipogenic maintenance medium (170 ml), adipogenic maintenance SingleQuots™ Kit, adipogenic induction medium (170 ml), and adipogenic induction SingleQuots™ kit
PT-3102B	Adipogenic induction medium	170 ml
PT-3102A	Adipogenic maintenance medium	170 ml
PT-4122	hMSC adipogenic maintenance SingleQuots™ kit	Frozen supplements and growth factors. (h-Insulin (recombinant), L-glutamine, mesenchymal cell growth supplement (MCGS), and GA-1000.)
PT-4135	hMSC adipogenic induction SingleQuots™ kit	Frozen supplements and growth factors. (h-Insulin (recombinant), L-glutamine, mesenchymal cell growth supplement (MCGS), GA-1000, dexamethasone, indomethacin and IBMX)
PT-7009	AdipoRed™ assay reagent	5 x 4.0 ml

### Differentiation media BulletKit™ – chondrogenic

PT-3003	hMSC differentiation BulletKit™ – chondrogenic	Contains differentiation basal medium – chondrogenic (185 ml) and hMSC chondrogenic SingleQuots™ kit.
PT-3925	Chondrogenic basal medium	185 ml
PT-4121	hMSC chondrogenic SingleQuots™ kit	Supplements and growth factors (ITS + supplement, dexamethasone, ascorbate, sodium pyruvate, proline, GA-1000, L-glutamine)

PT-4124	TGF-β3	1 vial of lyophilized TGF-β3, 2 μg (required chondrogenic differentiation reagent)
---------	--------	---

**NOTE:** TGF-β3 is supplied as a separate part from the hMSC chondrogenic SingleQuots™ kit

### Differentiation media BulletKit™ - osteogenic

PT-3002	hMSC differentiation BulletKit™ – osteogenic	Contains differentiation basal medium – osteogenic (170 ml), and hMSC osteogenic SingleQuots™
PT-3924	Osteogenic basal medium	170 ml
PT-4120	hMSC osteogenic SingleQuots™	Supplements and growth factors (dexamethasone, ascorbate, mesenchymal cell growth supplement (MCGS), L-glutamine, penicillin/streptomycin, β-glycerophosphate)
CC-3232	Trypsin/EDTA solution	100 ml

*human clinical trials; c) in association with human diagnostics.*

- MATERIAL NOT TRANSFERABLE:** You may not transfer the Material to any other person or organization
- PATENT NOTICE:** Material is under license from Osiris Therapeutics, Inc. Material is covered by US Patent 5,486,359 and others.

### Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza warrants its cells only if Poietics™ media are used, and the recommended protocols are followed. Cryopreserved hMSC are assured to be viable and functional when thawed and maintained properly.

### Quality control

HIV-1, hepatitis B and hepatitis C are not detected for all donors and/or cell lots. All cells are performance assayed and test negative mycoplasma, bacteria, yeast and fungi. Cell viability and morphology are measured after recovery from cryopreservation. For detailed information concerning QC testing, please refer to the certificate of analysis.

### Limited use license

Mesenchymal stem cells are produced for Lonza by Osiris Therapeutics, Inc. and are subject to the following limited use license:

The included biological material, including progeny and derivatives, (collectively referred to as “Material”) is licensed to you under specific terms. You are responsible for ensuring that the terms of the license agreement are met.

- GRANTS OF LICENSE:** Lonza grants you a nontransferable, nonexclusive license to use the Material for research.
- NOT FOR HUMAN USE:** The Material may not be used: a) in humans; b) in conjunction with