Tips for Using UltraGRO™ and UltraGRO™-Advanced to Grow Mesenchymal Stem Cells (MSCs)

HELIOS® Bioscience Brand, AventaCell Product, UltraGRO™/UltraGRO™-Adv. shows optimal growth of MSC at 5 % (v/v) in typical cell culture media, i.e. Alpha-MEM, which contains 2 mM L-Glutamine.

We recommend seeding MSCs at approximately 3×10^3 ~ 6×10^3 cells per cm^2.

For UltraGRO™ product, Heparin at a final concentration of 2 U/ml in the culture media supplemented with 5 % UltraGRO is required. Failure to add Heparin will cause coagulation during cell culture in typical medium. For UltraGRO™-Adv. product, addition of exogenous Heparin is **NOT** required.

UltraGRO™/UltraGRO™-Adv. Storage

UltraGRO™/UltraGRO™-Adv. product is most stable when stored frozen (-20°C) until needed.

Please thaw frozen UltraGRO™/UltraGRO™-Adv. product in a 37 °C water bath before use. Once UltraGRO™/UltraGRO™-Adv. product is thawed, it is recommended to use it for completed medium preparation (e.g. 5 %) immediately, or to divide it into single-use aliquots and store unused aliquots at -20 °C.

It is highly recommended to prepare the UltraGRO™/UltraGRO™-Adv. containing medium (e.g. 5 %) on the same day or one day before cell culture and store the unused UltraGRO™ containing medium at 2 ºC to 8 ºC no longer than 2 weeks.

Precipitation in Cell Culture

Clotting or insoluble particles may form in thawed UltraGRO™/UltraGRO™-Adv., it is recommended to centrifuge at 3,400 ×g for 3 ~ 5 minutes or to filter the liquid concentrate with a sterile 40 µm Cell Strainer to remove insoluble particles.

NOTE: 0.22 µm filtering is **NOT** recommended for UltraGRO™/UltraGRO™-Adv. 100 % concentrate.

Repeated freeze-thaw cycles should be avoided, as they will cause an increase in insoluble particles and potential decrease UltraGRO™/UltraGRO™-Adv. performance.

Isolation and Expansion of Bone Marrow- and Umbilical Cord Blood-derived MSCs

1. Preparation of Mononuclear cells (MNC) from bone marrow (BM): BM was isolated by aspiration from donors aged 20 to 30 years old (n=10), with informed consent. MNC were isolated from BM by loading onto Ficoll-Paque PLUS solution (d= 1.077) after 1:1 dilution with Dulbecco's phosphate-buffered saline (D-PBS).

   Preparation of MNC from umbilical cord blood (UCB): UCB was collected from umbilical veins after neonatal delivery with maternal informed consent (n ≥ 10). MNC were isolated from UCB units by loading onto Ficoll Hypaque solution (d= 1.077) after 1:1 dilution with Dulbecco's phosphate-buffered saline (D-PBS).

2. After centrifugation at 800×g for 15 ~ 20 minutes at room temperature, the MNC layer was removed from the interphase and washed twice with Dulbecco's phosphate-buffered saline (D-PBS).
3. Plate the MNC in non-coated 25 ~ 75 cm^2 polystyrene culture flasks (Corning) at a density of $1\sim2\times10^5/cm^2$ in complete culture medium.
4. Cultures were maintained at 37°C, in a 5% CO_2 humidified atmosphere. After 48 h, change the culture medium to remove non-adherent cells.
5. Culture medium was replaced twice a week.
6. Upon the appearance of MSC-like clones, cells were harvested using 0.25% trypsin, re-plated for expansion at a density of 3×10^3 ~ 6×10^3 cells/cm^2 and propagated in culture until reaching a senescence phase.
7. Cell growth was analyzed by direct cell counts and cumulative population doublings were determined.

Isolation and Expansion of Adipose-derived MSCs

1. Adipose tissue was obtained by liposuction from healthy donors aged 20 to 45 years (n ≥ 4), with informed consent.
2. Processed lipoaspirate (PLA) (15-30 ml) was washed with phosphate-buffered saline (PBS) buffer. Washed PLA was digested in PBS containing 0.2% collagenase (C-9891, Sigma) on a 37°C shaking water bath for 30 minutes.
3. Floating adipocytes were aspirated from pelleted AT-MSCs after centrifugation at 400 *g* for 10 minutes.
4. Pellets were resuspended in red blood cell lysis buffer (2.06 g/L Tris base, 7.49 g/L [0.1 mmol/L] NH4Cl, pH 7.2) for 10 minutes at room temperature.
5. After resuspension, the washed AT-MSCs were passed through a 40- to 100-mm cell strainer (Becton Dickinson, San Jose, CA). The washed cells were resuspended in growth medium Cells (1 x 10^6-2 x 10^6 cells/dish) were added into 10-cm culture dishes (Corning) and cultured at 37°C, in an atmosphere of 5% CO2 and humid air.
6. After 5 to 7 days, adherent cells were harvested as AT-MSCs by trypsinization and passaging into a new DMEM-LG culture medium containing the same supplements.
7. The medium was replaced every 3 to 4 days, when the cells reach near 80% confluence.

Preparation of the stromal vascular fraction (SVF)

Liposuction washing

1. Adipose tissue is collected by needle biopsy or liposuction aspiration and place the liposuction into a sterile medium bottle.
2. Allow the adipose tissue to settle above the blood fraction.
3. Remove the blood using a sterile 25 ml pipette.
4. Washing the tissue sample extensively by adding equivalent volume of phosphate-buffered saline (PBS) containing 5% Penicillin / Streptomycin (P/S) and shake vigorously for 5~10 sec.
5. Place the bottle on the bench and allow the adipose tissue to float above the PBS.
6. Carefully remove the PBS by using 25 or 50 ml pipette.
7. Repeat the above washing procedure (step 4 to 7) three times. (If the solution is still red, wash again by repeating step 4 to 7.)

Collagenase digestion

1. Place the washed tissue sample to T175 flasks and add 0.2% Collagenase Type I prepared in PBS containing 2% P/S for tissue digestion. The final volume required is half that of the washed adipose tissue volume.
2. Mince the adipose tissue sample using two scalpels and pipette the sample up and down with a 25 or 50 ml pipette several times to further facilitate the digestion. Incubate the sample for 1~2 hr at 37 °C, 5% CO2, and manually shaking the flasks for 5~10 sec every 15 min.
3. At the end of incubation, neutralize the Collagenase Type I activity by 5% UltraGRO™ or UltraGRO™-Advanced to a final concentration of 5%.
4. Pipette the sample up and down several times to further disintegrate aggregates of the adipose tissue.

Separation of the stromal-vascular fraction

1. Transfer the sample to a 50 ml tubes, avoiding the undigested tissue. The stromal vascular fraction (SVF), containing the ASCs, is obtained by centrifuging the sample at 800g for 10 min.
2. Aspirate the floating adipocytes, lipids and the digestion liquid, leaving SVF pellet in the tube.

Separation of stromal stem cells from the SVF

1. Resuspend the SVF pellet in 20 ml lysis buffer (2.06 g/L Tris Base, pH 7.2, 7.49 g/l NH_4Cl), incubate for 10 min at RT.
2. Centrifuge at 400xg for 10 and aspirate the supernatant.
3. Resuspend the pellet with 20 ml of PBS/2% P/S and centrifuged at 400xg for 10 min.
4. The supernatant is aspirated; the cell pellet is resuspended in a maximum of 3 ml of culture medium and pool the cell suspension into 50 ml tubes.
5. Filter cells through 100 µm cell strainers.
6. Pass and filter cells through 40 µm cell strainers.
7. Centrifuge at 400xg for 10 and aspirate the supernatant.
8. Resuspend cell pellet with culture medium and place cells in 25 ~ 75 cm^2 polystyrene culture flasks (Corning) at a density of $1~2\times10^5/cm^2$.
9. Cultures were maintained at 37°C, in a 5% CO_2 humidified atmosphere. After 24 h, change the culture medium to remove non-adherent cells.
10. Culture medium was replaced twice a week.

11. Upon the appearance of MSC-like clones, cells were harvested using 0.25% trypsin, re-plated for expansion at a density of 3×10^3 ~ 6×10^3 cells/cm^2 and propagated in culture until reaching a senescence phase.
12. Cell growth was analyzed by direct cell counts and cumulative population doublings were determined.