

Tips for Using UltraGRO™ to Grow Human Mesenchymal Stem Cells

HELIOS® Bioscience Brand, AventaCell Product, **UltraGRO™ (UG)** shows optimal growth of human Mesenchymal Stem Cells (hMSCs) 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 $3 \times 10^3 \sim 6 \times 10^3$ cells per cm^2 .

In the culture media supplemented with UG, **addition of exogenous Heparin at a final concentration of 2 U/ml is required.** Failure to add Heparin will cause coagulation during cell culture in typical medium.

Storage

UG is most stable when stored frozen (-20 °C).

Usage

Please thaw frozen UG in 37 °C water bath before use. Once UG is thawed, remove from water bath immediately. It is **NOT** recommended to thaw UG at lower temperature (e.g. 4 °C or RT) demanding longer thawing time, which may cause an increase in number/size of insoluble particulates and potentially compromise UG potency.

It is recommended to use thawed UG for complete 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 UG containing medium on the same day or one day before cell culture and store the unused UG medium at 2 °C to 8 °C no longer than 2 weeks.

Precipitation in Cell Culture

Clotting or insoluble particles may form in thawed UG. Before applying thawed UG in culture medium preparation, it is recommended to centrifuge at $3,400 \times g$ for 3 ~ 5 minutes or to filter with a sterile 40 μm Cell Strainer to remove insoluble matter.

Alternatively, applying 0.22 μm filter to the completed UG medium (e.g. 5%) will not affect the cell culture performance.

Note: 0.22 μm filtering is **NOT** recommended for UG 100 % concentrate.

Repeated freeze-thaw

Although UG can sustain a few cycles without compromising the potency, repeated freeze-thaw should be avoided, as they will enhance the number/size of insoluble particulates and potentially lose the vital factors for cell culture performance.