

Tips for Using UltraGRO™ to Grow Mesenchymal Stem Cells

HELIOS® Bioscience Brand, AventaCell Product, UltraGRO™ shows optimal growth of MSCs 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 UltraGRO™, **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

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

Usage

Please thaw frozen UltraGRO™ in 37 °C water bath before use. Once UltraGRO™ is thawed, remove from water bath immediately. It is **NOT** recommended to thaw UltraGRO™ 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 UltraGRO™ potency.

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

Note:

Precipitation in Cell Culture

Clotting or insoluble particles may form in thawed UltraGRO™. Before applying thawed UltraGRO™ in culture medium preparation, it is recommended to centrifuge at 4,000 $\times g$ for 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 UltraGRO™ medium (e.g. 5%) will not affect the cell culture performance.

- 0.22 μm filtering is **NOT** recommended for UltraGRO™ 100 % concentrate.

Repeated freeze-thaw

Although UltraGRO™ 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.