

Tips for Using UltraGRO™-PURE GI in Closed System Bioprocess to

Grow Mesenchymal Stem Cells

HELIOS® Bioscience Brand, AventaCell Product, **UltraGRO™-PURE GI (UG-P GI)** shows optimal growth for mesenchymal stem cells (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 UG-P GI, **addition of exogenous Heparin is NOT required.**

Storage

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

Usage

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

Apply adequate connectors such as Luer lock male, MPC female, or the adaptor to 3/4" tri-clamp sanitary flange (for 1L bag product connection only) to the bag's port for further use. It is recommended to use thawed UG-P GI 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-P GI containing medium on the same day or one day before cell culture and store the unused UG-P GI 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-P GI. Before applying thawed UG-P GI in culture medium preparation, it is recommended to centrifuge at 3,400 $\times g$ for 5 minutes to remove insoluble matter. Alternatively, applying 0.22 μm filtration to the prepared UG-P GI containing medium (e.g. 5%) will not affect the cell culture performance.

Note: 0.22 μm filtration is **NOT** recommended for 100% UG-P GI concentrate.

Repeated freeze-thaw

Although UG-P GI 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.