

## Tips for Using UltraGRO™-Advanced to Grow Human Mesenchymal

### Stem Cells

HELIOS® Bioscience Brand, AventaCell Product, **UltraGRO™-Advanced (UG-A)** shows optimal growth of Grow 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 hMSCs at approximately  $3 \times 10^3 \sim 6 \times 10^3$  cells per  $\text{cm}^2$ .

In the culture media supplemented with UG-A, **addition of exogenous Heparin is NOT required.**

### Storage

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

### Usage

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

It is recommended to use thawed UG-A 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-A containing medium on the same day or one day before cell culture and store the unused UG-A 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-A. Before applying thawed UG-A in culture medium preparation, it is recommended to centrifuge at  $3,400 \times g$  for 3 ~ 5 minutes to remove insoluble matter. Alternatively, applying 0.22  $\mu\text{m}$  filtration to the prepared UG-A containing medium (e.g. 5%) will not affect the cell culture performance.

**Note:** 0.22  $\mu\text{m}$  filtration is **NOT** recommended for 100% UG-A concentrate.

### Repeated freeze-thaw

Although UG-A 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.