Aging with VitroAge

Traditional keratinocyte cell culture media actively resist the aging process with a variety of anti-aging, protective, and highly-stimulatory ingredients. As a result, they are a poor foundation for in vitro aging studies.

In contrast, the VitroAge medium CnT-AG2 does not contain the concentrated range of anti-aging ingredients found in standard media. Keratinocytes grown in CnT-AG2 are not exposed to these protective factors, and as a result develop a range of age-related changes during several weeks of culture.

Key changes observed in keratinocytes cultured in CnT-AG2 include decreased longevity, progenitor cell activity, DNA repair, protein synthesis, and antioxidant/stress response mechanisms. In parallel a number of cellular functions increase, including protein catabolism, protein carbonylation, and detoxification mechanisms. Aged keratinocytes can also be evaluated using a novel multiple proteomic approach that characterizes approximately 100 age-related proteins in parallel. For more information please visit: https://cellntec.com/wpcontent/uploads/pdf/IID_Poster_CELLnTEC_A3.pdf.

Protocol Considerations

Duration: The standard VitroAge protocol involves culturing keratinocytes for a period of 3 to 4 weeks in either CnT-Prime (control) medium, CnT-AG2, or CnT-AG2 plus an active ingredient. Cell counts are conducted at the end of each week, to allow evaluation of the comparative proliferation rate of each treatment. Cells may be sampled for evaluation of age-related markers after 2 or more weeks of culture, depending on the particular end-point of interest.

Cells: We recommend the use of early passage keratinocytes isolated from young donors (children or young adult). These keratinocytes should be growing well in CnT-Prime medium prior to transfer to the CnT-AG2 medium, to ensure that the cells will easily tolerate the transfer (transfers between un-related media can be very challenging).

Passaging: We strongly recommend the use of gentle digestion reagents such as Accutase (#CnT-Accutase-100) when passaging keratinocytes aged in CnT-AG2. These enzymes are far gentler than the aggressive digestion obtained with enzymes such as Trypsin. Accutase does not require a separate reagent to stop the reaction, just dilution prior to centrifugation. We recommend diluting, centrifuging, and counting aged cells in control medium.

Centrifugation: Keratinocytes grown in CnT-AG2 remain very sticky after detachment for passaging. They adhere rapidly to the walls of the centrifuge tubes, and then do not end up in the cell pellet which significantly reduces harvest and growth following re-seeding. For this reason, it is critical that 0.1% serum albumin is included in the cell solution immediately after detachment, to prevent loss in the centrifuge tube.

Seeding: The standard seeding density of 4'000 cells / cm^2 can be lowered during the first passage of culture to allow for weekly counts. As the proliferation rate gradually slows in CnT-AG2 during weeks 2 to 4, the seeding density may be increased gradually (for example to 8'000 cells per sq cm), to obtain a larger number of cells for sampling at the end of the aging period. Alternatively, or additionally also the culture surface can be increased over time.

We strongly recommend seeding the cells in control medium throughout the experiment and switching to CnT-AG2 only the next day for the remaining time of each passage.

Timing: We recommend 3 medium changes per week (Mon / Wed / Fri) with a medium change prior to the day of passaging.

For further information about the standard culture protocols we recommend to use with CELLnTEC media, please visit the protocols page: http://www.cellntec.com/products/resources/protocols

For other technical questions, please contact our scientific team: support@cellntec.com