Directions for AQIX® Cryopreservation of HS-cells [SOP/HS/CRYO]

Step 1. Harvest HS-cells at 10^7 to 10^6 cells/mL.

Step 2. Prepare pellets of HS-cells at 10^7 to 10^6 cells/mL by gentle centifugation in suitable vials.

Step 3. Place HS-cell pellet vials, unopened containers of sterile AQIX® RS-I/1G solution and cryopreservative vials (e.g., NUNC™ Cryoflex) into a laminar flow cabinet.

Step 4. *Gently* add the cryopreservative fluid, AQIX® RS-I/1G to the HS-cell pellets to acheive 10^7 to 10^6 cells/mL suspension volumes of the cryopreserve fluid to a maximum of 1-2 mL volumes.

Step 5. Allow HS-cell suspensions to equilibrate at ambient temperatures for 15 – 30 minutes within the laminar flow cabinet.

Step 6. Place 1-2 mL volumes of the HS-cell suspension media into suitable cryopreservation vials (e.g., NUNC™ Cryoflex) and seal tightly to ensure that no leakage of Nitrogen gas into the vials occurs during cooling and storage.

Step 7. Remove sealed, cryopreservation viles from the laminar flow cabinet and transfer to a suitable cryopreservation system (e.g., Nalgene "Mr. Frosty"; Cat.No. 5100-0001).

Step 8. Cool HS-cell suspension vials at 1°C/min to -40 °C and then transfer into a suitable storage unit to achieve the final, desired cropreservation temperature (e.g., -60 °C to -150 °C).

Step 9. After 48 hours of cryopreservation, remove a sample vial to test for cell viability. Proceed as directed in Step 10.

Step 10 To reconstitute cryopreserved HS-cells, allow the frozen vials of suspension media to defrost at 37 °C in a tissue culture cabinet or (less preferred) within a conventional water bath.

Step 11 *Gently* pipette off HS-cell suspension and transfer 1.0 mL of the suspension into either (a) 2.0 mL of (1x) AQIX® RS-I solution or (b) 2.0 mL of a preferred TC-media. Repeat this procedure a further two times to dilute out the AQIX® RS-I/1G cryopreservation fluid.

Step 12. Test HS-cell viability using conventional procedures.