

AQIX[®] FLUID TECHNOLOGY - APPLICATION OF CARBOGEN GAS

1. AQIX[®] STORAGE SOLUTIONS

- a) Previous trials have shown that optimal preservation of the functionality of isolated animal and human biopsy samples are achieved when AQIX[®] RS-I and RS-S solutions are pre-aerated with *carbogen* gas [95% O₂ / 5% CO₂].
- b) *Carbogen* gas cylinders come in 10L and 40L sizes – 10L will suffice in this application. A 2 – 4 psi ‘Regulator’ valve will be required and set to 1-2 psi to deliver adequate flow of the *carbogen* gas.
- c) The carbogenation of AQIX[®] RS-I and RS-S solutions is achieved by simply passing *carbogen* gas (e.g., via a tube plus attached 20-22G syringe needle) through a 0.22µm filter into these solutions for 15 – 30 minutes [within a suitable, gas impermeable container] and then tightly sealing the lid closure of the container.
- d) The sealed, gassed containers can be stored for up to 6 weeks at 3 – 8 °C under dark conditions prior to being dispatched to the tissue biopsy procurement site.
- e) The carbogenated AQIX[®] RS-I and RS-S solutions can be transported to the procurement site over ‘wet’ ice [0 - 4 °C] or at ambient temperatures [< 25 °C].
- f) At the procurement site, the sealed containers are opened and the tissue biopsy sample inserted and the containers re-sealed quickly to prevent the outward diffusion of oxygen and carbon dioxide.
- g) The sealed containers of either AQIX[®] RS-I and RS-S solutions are then transported back to the investigative laboratory over ‘wet’ ice [0 - 4 °C] or at ambient temperatures [< 25 °C].
- h) Transportation times of 12 – 96 hours are achievable for biopsies stored over ‘wet’ ice [0 - 4 °C] and 12 – 24 hours for biopsies stored at ambient temperatures [< 25 °C].
- i) At the investigative laboratory, the tissue biopsies should be examined immediately using (i) normothermic, isolated tissue perfusion techniques [see Section 2] or (ii) incubated under conventional Tissue Culture conditions **but** using **humidified, carbogen gas** at 37 °C [see Section 3].

2. AQIX[®] RS-I PERFUSION SOLUTION

- a) Tissue biopsy samples previously stored in AQIX[®] RS-I or RS-S solutions are re-animated using *carbogenated* AQIX[®] RS-I solution during the continuous perfusion of AQIX[®] RS-I at 1 – 4 mL/min at 32 - 38 °C.
- b) AQIX[®] RS-I solution is placed in a suitable reservoir container and then continuously aerated with *carbogen* gas during the total experimental time.

- c) The AQIX[®] RS-I solution within the experimental tissue chamber or bath is continuously *carbogenated* and replenished with fresh, *carbogenated* AQIX[®] RS-I solution at 1 – 4 mL/min at 32 - 38 °C.
- d) Tissue preparations may be examined under ‘non-perfused’ conditions (e.g., drug applications) at 32 - 38 °C for up to 30 minutes while maintaining continuous aeration with *carbogen* gas and then returned to perfused conditions and allowed 15 – 30 minutes to re-equilibrate.

3. AQIX[®] SOLUTIONS UNDER TISSUE CULTURE CONDITIONS

- a) AQIX[®] RS-I /RS-S/RS-C perfusion/storage solutions can be used under TC-conditions but require to be incubated using humidified, 5% Carbon Dioxide gas, in order to establish the P_{CO_2} / HCO_3^- buffering level at 37 °C to maintain a pH of 7.42 ± 0.04 .
- b) AQIX[®] fluids require to be replenished every 24 hours with fresh fluids.
- c) AQIX[®] fluids require to be stored at 3 - 8 °C under dark conditions prior to use.

4. General Comments

4.1 Pre-aerated AQIX[®] RS-I and RS-S solutions are packaged in sealed, non-diffusible containers and ‘ready-to-use’ at the site of tissue procurement.

4.2 Assembly of ‘ready-to-use’ AQIX[®] RS-I and RS-S solutions from 100mL vials of [10x] concentrates requires the simple addition of 2.1g/L of Sodium Hydrogen Carbonate and 900 mL of Ultra Pure water [refer ‘Product’ insert]