



INSTRUCTIONS FOR THE ASSEMBLY AND USE OF AQIX[®] RS-I SOLUTION

1. Dilution of the 10X concentrate solution to 1X 'ready-to-use' solution
2. Dispensing of 1X solution into containers
3. Shelf life of the solutions
4. Use of the 1X solution containers for transport
5. Use of the 1X solution of for perfusion and culture

RELEVANT ADVICE

It is important to note that AQIX[®] RS-I is a unique, synthetic formulation which has been designed to reproduce and act in the same way as human interstitial fluid. It has been formulated according to strict scientific principles:

- 1) With the exception of one key analogue [BES], there is nothing in AQIX RS-I that is not found in the human body.
- 2) All ingredients in the formulation are within the concentration ranges found in human serum and interstitial fluid.
- 3) All ingredients are included for one or more known and specific purposes.
- 4) All ingredients and their concentrations have been tested to ensure that they are essential and sufficient for the maintenance of essential metabolic pathways.

AQIX[®] RS-I achieves vastly superior maintenance of cells, tissues and organs, using mechanisms very different from conventional, preservation and suspension media. In order for these mechanisms to be effective it is essential to follow this guidance rather than adopt other procedures which may be more familiar, but which have been proven to be inappropriate.

By way of example, AQIX[®] RS-I creates an analogue of part of the natural, human serum pH buffering mechanism, namely, the partial pressure of carbon dioxide dissolved in a solution containing hydrogen carbonate ions, in preference to using phosphate ions, which have been shown to cause deleterious and irreversible alterations in cell structure and numerous biochemical processes within a few hours of exposure. Part of this guidance is aimed at creating and sustaining the natural, pH buffering mechanism.

A second important consideration is that tissues stored in AQIX[®] RS-I remain metabolically active, even under hypothermic conditions, and will continue to consume the essential ingredients of this solution. This fact, combined with the principle of only using natural 'serum' level concentrations of substrates in the solution, means that greater volumes of the solution may be required to ensure that the tissues remain bathed in their natural, intercellular environment and do not become depleted of metabolic substrates during storage.

The following information is intended to assist the operator in the correct utilisation of AQIX[®] RS-I storage/transportation/perfusion solution. Good laboratory practice (GLP) must be adhered to throughout the complete process.

1. Dilution of the 10X concentrate to a 1X solution

| Step | Details | Completed | Variation |
|---|--|-----------|-----------|
| 1.1 Assemble necessary equipment | Place the following into the hood of a laminar flow cabinet | | |
| | 1.1.1 100ml bottle of AQIX [®] RS-I 10X concentrate solution | | |
| | 1.1.2 1L sterile, stoppered, graduated volumetric flask | | |
| | 1.1.3 Weighing boat containing 2.10 g of Sodium Hydrogen Carbonate [NaHCO ₃ ; Sigma 4019] | | |
| | 1.1.4 Sterile 10 cm diameter filter funnel | | |
| | 1.1.5 0.2µm filter (optional) | | |
| | 1.1.6 Volume of Milli-Q [®] purified water [or equivalent ATSM Type I; 18.2 MΩ at 25 °C] sufficient to provide 900 mL for 1:10 dilution of AQIX [®] RS-I 10X concentrate solution. | | |
| | 1.1.7 Absolute alcohol and swabs. | | |
| | 1.1.8 Suitable container for the assembled solution (see Section 2) | | |
| 1.2 Commence procedure | Fill the 1L sterile, volumetric flask with approximately 500mL of the purified water | | |
| 1.3 | Place the sterile 10 cm diameter filter funnel into the 1L flask. | | |
| 1.4 Clean, remove and rinse stopper. | Swab the plastic and metal cap of the AQIX [®] RS-I 100 mL bottle with alcohol and then carefully remove metal cap and rubber seal from bottle. Rinse the rubber seal briefly with the purified water into the funnel and thereby into the 1L volumetric flask. | | |
| 1.5 Empty and rinse AQIX [®] RS-I 100 mL bottle. | Pour the contents of the bottle <u>carefully</u> into the funnel, then rinse the bottle three times with a portion of the purified water, also into the funnel. | | |
| 1.6 Add 2.10 g of Sodium Hydrogen Carbonate | Add the 2.10 g of Sodium Hydrogen Carbonate and rinse with the purified water into the funnel | | |
| 1.7 Rinse funnel and top up volume. | Rinse the funnel into the flask with purified water and remove from neck of the volumetric flask. | | |

| Step | Details | Completed | Variation |
|--|--|-----------|-----------|
| 1.8 | Top up the 1L volumetric flask with purified water to the graduation mark. | | |
| 1.9 Mix and dissolve the sodium hydrogen carbonate | Invert stoppered 1L flask and shake the contents until Sodium Hydrogen Carbonate has completely dissolved (approx. 5 minutes) <u>without</u> heating the AQIX [®] RS-I solution in the flask. | | |
| 1.10 Dispense according to Section 2. | The 1L of AQIX [®] RS-I solution may also be finally filtered via a 0.2µm filter into a suitable sealed container. | | |
| 1.1110 Note on making up quantities less than 1L | <p>Smaller quantities of AQIX[®] RS-I solution 1X solution can be made up from the 10X concentrate solution.</p> <p>Remove inner metal tag and insert a sterile syringe needle through the rubber bung and remove the required quantity of 10x concentrate (e.g., 10mL).</p> <p>Follow the procedures above adjusting the amount (e.g., 0.21g) of Sodium Hydrogen Carbonate accordingly.</p> | | |

2. Dispensing and Storage of AQIX® RS-I 1X solution

| Step | Details | Completed | Variation |
|---|--|-----------|-----------|
| 2.1 Storage container requirements | Storage containers must prevent the gain or loss of carbon dioxide, oxygen and water from the prepared solution. Commonly used materials such as polypropylene will not do this. | | |
| 2.2. Acceptable storage containers | 2.2.1 Glass containers with an airtight closure. | | |
| | 2.2.2 Suitable plastic polymer [D _{CO2} < 20 cm ³ /m ² @ bar/24hr] | | |
| | 2.2.3 Examples: Nalgene PETG containers such as product codes 2019-0125 Sterile Square Media bottle 2035-0005 5ML PETG bottle | | |
| | | | |
| 2.3 Dispensing instructions for transport bottles | Place the following into the hood of laminar flow cabinet | | |
| | 2.3.1 Bulk container of 1x AQIX® RS-I solution to be dispensed | | |
| | 2.3.2 Supply of <i>carbogen</i> gas [i.e. 95% O ₂ / 5% CO ₂] via polyethylene tubing with in-line 0.2µm filter | | |
| | 2.3.3 0.2µm filter | | |
| | 2.3.4 Suitable storage containers | | |
| 2.4 Commence Procedure | Bubble the carbogen gas through the bulk liquid via a sterile, 1 mL pipette for approximately 15-20 mins per litre to achieve pH of 7.26 ± 0.04 @ 20 °C. | | |
| 2.5 Dispense through sterile filter | Filter via sterile 0.2µm filter unit into the containers. | | |
| 2.6 Attach stoppers promptly | Ensure that closures are firmly attached immediately after filling. | | |
| 2.7 Apply Label | Label appropriately, immediately after dispensing, including expiry date (See Section 3). | | |

3. Shelf life of the solution

| Step | Details | Completed | Variation |
|---|--|-----------|-----------|
| 3.1 Storage conditions | 3.1.1 Temperature +3 to +8 Degrees Celsius 3.1.2 Store in the dark, away from all light sources. | | |
| 3.2 10X Solution | Unopened 100mL bottles of 10x concentrate AQIX [®] RS-I solution will have a shelf life in excess of 14 months from manufacture (see expiry date on bottle). (Must be stored as directed) | | |
| 3.3 Bulk 1X | 1x solutions of AQIX [®] RS-I can be kept for up to twelve weeks when stored as directed in sealed, suitable containers (see Section 2) | | |
| 3.4 Carbogentated 1X | 1x solutions of <i>carbogentated</i> AQIX [®] RS-I solutions can be kept for up to twelve weeks when stored as directed in sealed, suitable containers. | | |
| 3.5 AQIX [®] RS-I Kits (product code RSIKIT) | AQIX [®] RS-I Kits (RSIKIT) can be kept for up to twelve weeks when stored in sealed, suitable containers as directed. If unopened they do not require carbogenation. | | |
| 3.6 Precipitates | Correctly assembled and stored AQIX [®] RS-I solution will show no precipitates. | | |
| 3.7 Contamination | Do not use any solution that shows turbidity, precipitates or any other sign of contamination. | | |

4. Use of the 1X containers for transport

| Step | Details | Completed | Variation |
|---------------------------------|---|-----------|-----------|
| 4.1 Containers | Transport containers should be purchased from the manufacturer's agent or prepared according to the instructions above (Section 2) | | |
| 4.2 Storage | Containers should be stored according to the instructions above (Section 3). | | |
| 4.4 Sample size to volume ratio | The volume of solution needed depends upon: 1) Tissue type 2) Transport temperature 3) Transport time | | |
| 4.5 Basic Guidance | For normally active tissue transported for up to 36 hours at 4 degrees celsius a ratio of 50ml of Aqix RS-I for each 1g of tissue has been shown to be effective. | | |
| 4.6 Additional Guidance | Additional information relating to experience of various tissue types, transport times and temperatures can be found at www.aqix.com | | |
| | | | |
| 4.7 Transport Procedure | 4.7.1 Minimise time that the solution is exposed to light | | |
| | 4.7.2 Minimise the time that the lid closure is removed from the container. | | |
| | 4.7.3 It is better to place the entire tissue immediately into the container rather than attempt any cleaning or other procedure which may prolong the period during which the tissue is outside of its natural environment (subject to the volume ratio considerations of 4.4) | | |
| | 4.7.4 Transport at 0-4 degrees Celsius (wet ice) using a buffer layer of cold water between the bag containing the sample bottle and the container of ice if there is any danger of the ice (or cool packs) cooling the sample below Zero degree Celsius. | | |

5. Use of the 1X solution of for perfusion and culture

| Step | Details | Completed | Variation |
|--|--|-----------|-----------|
| 5.1 | Aqix has been used extensively for the long term perfusion of mammalian/human tissues and organs. For detailed information about tissue types and conditions please consult www.aqix.com or contact customer services on the numbers given on the website. | | |
| 5.2 General principles for perfusion and culture | 5.1 Aqix RS-I can carry oxygen and carbon dioxide suspended in the solution sufficient for many hypothermic and normothermic perfusion applications. | | |
| | 5.2 Continuous aeration of AQIX [®] RS-I solution with <i>carbogen</i> gas [i.e. 95% O ₂ / 5% CO ₂] is recommended during perfusion experiments | | |
| | 5.3 Care must be given to ensure adequate replenishment of the solution during an experiment to replace substrates being actively consumed. | | |
| | 5.4 Test substances, blood cells, cytokines, antibiotics and other substances can be added to AQIX [®] RS-I solution provided that they are not added at inhibitory concentrations. | | |
| | 5.5 The levels of drugs, metabolites, metabolic by-products, etc., have been successfully analysed in the AQIX RS-I perfusate over 6 – 12 hours during normothermic perfusion of animal/human tissues and organs (www.aqix.com) | | |
| | 5.6 The pH buffering mechanism is effective across a range of temperatures from hypothermic to normothermic. | | |

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