



INSTRUCTIONS FOR THE ASSEMBLY, STORAGE AND PERFUSION OF AQIX[®] RS-I (1x) 'Ready-to-Use' Solution USED IN *Ex Vivo* TISSUE AND ORGAN RESEARCH

It is important to note that AQIX[®] RS-I is a NON phosphate pH buffered solution. Therefore, *ex vivo* experimentation, the dilution, preparation, and utilisation processes for AQIX RS-I are different to conventional phosphate-buffered saline solutions.

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

Point 1: To assemble a 1L volume of AQIX[®] RS-I solution from 100 mL of 10x concentrate AQIX[®] RS-I

Directions:

- i. Half fill a 1L sterile, volumetric flask with approximately 500 mL of Milli-Q[®] purified water [or equivalent ATSM Type I; 18.2 M at 25 °C].
- ii. Place a sterile 10 cm diameter filter funnel into the 1L flask.
- iii. Carefully remove the plastic cap, metal cap and then rubber seal from the 100 mL bottle of 10x concentrate AQIX[®] RS-I solution. Rinse the rubber seal briefly with Milli-Q[®] purified water into the funnel and thereby into the 1L volumetric flask.
- iv. Now pour the contents of the 100 mL bottle of AQIX[®] RS-I 10x concentrate carefully into the funnel, then rinse the bottle three times with Milli-Q[®] purified water, also into the funnel.
- v. Weigh out 2.10 g of Sodium Hydrogen carbonate [NaHCO₃; Sigma 4019] and rinse with Milli-Q[®] purified water into the funnel.
- vi. Finally, rinse the funnel into the flask and top up to the graduated 1L mark on the volumetric flask with Milli-Q[®] purified water.
- vii. Invert stoppered 1L flask and shake the contents until NaHCO₃ has completely dissolved (approx. 5 minutes) without heating the AQIX[®] RS-I solution in the flask.
- viii. Aerate with *carbogen* gas [ie. 95% O₂ / 5% CO₂] to give a pH of 7.22 ± 0.04 @ 20 °C (approx 10 -15 minutes).
- ix. Transfer the 'Ready-to-Use' AQIX[®] RS-I solution to suitable perfusion equipment (e.g. Res-Del[®] Perfusion System/ORS *Ex Vivo* System), and continuously aerate with carbogen gas during the perfusion of isolated tissue and organ preparations.

Point 2: Storage of AQIX[®] RS-I solution:

- i. Unopened bottles of 10x concentrate AQIX[®] RS-I solution will have a shelf life in excess of 14 months. (Must be stored as directed, below 8 °C).

- ii. Prior to being carbogenated, 1x concentrate solutions of AQIX[®] RS-I can be kept for up to twelve weeks when stored in sealed containers at 3 - 8 °C. (Must be prepared using good laboratory practice). When diluted and stored as directed, there should be NO precipitation.
- iii. 1x concentrate solutions of *carbogenated* AQIX[®] RS-I solutions can be kept for up to six weeks when stored in sealed containers at 3 - 8 °C.. (Must be stored in sealed containers). When diluted and stored as directed, there should be NO precipitation.

Point 3: Preparation of 10 - 100 mL aliquots of AQIX[®] RS-I solution:

Small 1 - 10 mL volumes of the 10x concentrate AQIX[®] RS-I solution can be used to make up 1 - 10 mL of AQIX[®] RS-I by simply withdrawing 1 - 10 mL from the sealed bottles under sterile conditions, diluting with a proportional volume of Milli-Q[®] water and then adding 0.021 - 0.21g respectively of NaHCO₃ [Sigma 4019] to give a pH of 7.22 ± 0.04 @ 20 °C.

Point 4: Aeration during use:

For tissue or organ perfusion, continuous aeration of AQIX[®] RS-I solution with *carbogen* gas [i.e. 95% O₂ / 5% CO₂] is recommended.

Point 5: Procedures for the Perfusion of Tissue/Organ Biopsies:

A. Perfused Organ Preparations

- a) Prepare adequate volumes of AQIX[®] RS-I solution and fill the perfusate reservoir in order to prime the various equipment components of the perfusion system prior to the perfusion of the isolated organ preparation.
- b) Pre-aerate AQIX[®] RS-I solution in the perfusate reservoir for 10 – 20 minutes with *carbogen* gas [95% O₂ / 5% CO₂] to achieve a pH of 7.26 ± 0.04 @ 20 °C.
- c) Flush the aerated AQIX[®] RS-I solution through the perfusion equipment to the point of attachment into the vasculature of the isolated organ.
- d) Place the isolated organ preparation into the organ perfusion chamber and attach the respective cannula (e) to the vasculature of the organ (e.g., hepatic artery and portal vein).
- e) Activate the heating equipment and commence the continuous, normothermic perfusion of the isolated organ with carbogenated AQIX[®] RS-I solution.
- f) An equilibration period of 15 – 30 minutes is required prior to the examination of physiological and pharmacological events.

B. Perfused Tissue Preparations

- a) Prepare adequate volumes of AQIX[®] RS-I solution and fill the perfusate reservoir in order to prime the various equipment components of the perfusion system prior to the perfusion of the isolated tissue biopsy preparation.
- b) Pre-aerate AQIX[®] RS-I solution in the perfusate reservoir for 10 – 20 minutes with *carbogen* gas [95% O₂ / 5% CO₂] to achieve a pH of 7.26 ± 0.04 @ 20 °C.
- c) Flush the aerated AQIX[®] RS-I solution through the perfusion equipment and into

the isolated tissue preparation chamber.

- d) Place the isolated tissue preparation into the perfusion chamber and secure to the respective modules of the recording equipment (e.g., force/pressure transducers).
- e) Activate the heating equipment and commence the continuous, normothermic perfusion of the isolated tissue with carbogenated AQIX® RS-I solution.
- f) An equilibration period of 15 – 30 minutes is required prior to the examination of physiological and pharmacological events.

Point 6: Important information

Comparing AQIX® RS-I to other perfusion solutions.

Most perfusion solutions are phosphate buffered. Although there may be some apparent short-term benefit, the presence of inorganic phosphate ions has been shown to cause deleterious and irreversible alterations in cell structure and numerous biochemical processes within a few hours.

One of the unique, patented features of AQIX® RS-I solution is the absence of inorganic phosphate ions as a pH buffering agent.

It is therefore vital to ensure that in any comparative test no trace contamination of phosphate ions exists by replacing all containers, line-feeds and other apparatus when changing to AQIX® RS-I perfusate.

For further information on this preservation and perfusion solution and other AQIX® solution products refer to www.aqix.com or contact:

Aqix Ltd
Imperial College London, Incubator
London, SW7 2AZ, UK
Technical: quality@aqix.com
Orders: sales@aqix.com
Tel: +44 20 7193 8555

Manufactured for Aqix Ltd by:
NuPharm Laboratories Limited, CH5 2NT, UK