

AQIX® RS-I Medium: Technical Information

Overview

AQIX® RS-I is a sterile, chemically defined and optimised media developed to provide a stable storage and shipping environment for both tissues and cells. AQIX is fully defined and serum and animal/human protein free. The media may be used at a variety of temperatures (eg: 2°C to 37°C), creating an homeostatic environment for maintaining isolated human cells, tissues and organs over hours or days, as required.

AQIX® RS-I utilises a novel, acid-base (pH) buffering system designed to mimic the natural, α -stat pH mechanism of the imidazole groups in haemoglobin operating in mammals and humans and provides an ideal fluid composition to retain the acid-base balance and integrity of human cells and tissues, thereby avoiding the issues of necrosis and apoptosis in cell and tissue samples post collection.

- The AQIX® RS-I is an immediately 'ready-to-use' transport and storage medium for use for the collection of a wide range of cells and tissue samples.
- AQIX® RS-I can be used under both 'cold' and/or 'body' temperature conditions – a unique feature not available from formulations from other vendors.
- AQIX® RS-I has been designed to be isosmotic, isotonic and isoionic with human serum and interstitial fluid.
- AQIX® RS-I utilises a novel, acid-base (pH) buffering system designed to mimic the natural, α -stat pH mechanism of the imidazole groups in haemoglobin operating in mammals and humans and provides an ideal fluid composition to retain the acid-base balance and integrity of human cells and tissues.

Advantages

Cell support, storage and transportation

- Cell storage and transportation times of 12 - 120 hours under 'cold' (0 - 4 °C) or ambient temperatures
- Stable pH-buffering of pH 7.20 - 7.45 over 20 - 37 °C
- Reproducibility of protocols
- Ability to 'hold' stem cells during storage and delivery in differentiated format
- Operates under aerobic or anaerobic conditions to accommodate all stem cell types
- Suitable medium for use in cell culture containers currently used for transportation
- Animal and Human serum free
- Endotoxin levels conform to EU standards (≤ 0.025 EU/mL)
- Solvent free e.g. DMSO-free
- May be used to support all types of animal and human cells
- Suitable for washing cells in a variety of cell culture applications

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Organs

- Isosmotic and isionic to serum and interstitial fluid
- Suitable for the maintenance of functionality of organs
- Ideal as an organ flush fluid
- Suitable as organ support medium for the support of organs under normothermic conditions
- May be used to support all types of animal and human organs

Tissues

- May be used to support all types of animal and human tissue
- Ideal for functional support of fresh biopsied cells and tissues for up to 72 hours
- Does not compromise genetic and histological profiles of cells or tissues

Presentation

AQIX® RS-I is supplied as a sterile ready-to-use solution. AQIX® RS-I is available with antibiotic: Amphotericin B and Chloramphenicol or Amphotericin B and Nanomycopulitin.

Product Code	Product Description	Pack Size
AQIX-RSI-P	AQIX® RS-I Cell and Tissue Transportation Medium 'Ready to use'	125 mL
AQIX-RSI-P-AA	AQIX® RS-I Cell and Tissue Transportation Medium 'Ready to use' with Antibiotic (Amphotericin B and Chloramphenicol)	125 mL
AQIX-RSI-P-AN	AQIX® RS-I Cell and Tissue Transportation Medium 'Ready to use' with Antibiotic (Amphotericin B and Nanomycopulitin)	125 mL
AQIX-RSI-L	AQIX® RS-I Cell and Tissue Transportation Medium 'Ready to use'	1 litre
AQIX-RSI-10x-B	AQIX® RS-I Cell and Tissue Transportation Medium [10X] Concentrate	Available on request for bulk users

Product Information

Cell support, storage and transportation

AQIX® RS-I provides an ideal fluid composition to retain the acid-base balance and integrity of human cells, e.g., lymphocytes, erythrocytes, hepatocytes, cardiomyocytes, both inside and outside the body. The unique features of this formulation facilitate mitochondrial metabolism in the generation of the basic energy molecule, adenosine triphosphate (ATP), without which, all cells die. Red blood cells (RBC's) do not contain mitochondria to generate ATP, so AQIX® RS-I also contains thiamine pyrophosphate, which interacts to produce ATP resulting in increased longevity of these oxygen-haemoglobin carrying cells.

AQIX® RS-I satisfies the criteria necessary for use as a preservation storage and transportation medium with the additional benefit that, unlike other commercial products, it is suitable for a myriad of stem cell

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and peripheral blood cell types without the addition of disruptive compounds (e.g., DMSO) for preservation of cell function under 'cold' (hypothermic) or body (normothermic) temperatures, the latter being of specific interest for use in cell culture bioreactors.

Preliminary work has demonstrated that the addition of AQIX[®] RS-I gives a boost to the speed of cell division which is particularly interesting for cells with a higher respiration rate such as HEPG2 or monoclonal antibodies. In addition to giving a boost to the culture, AQIX[®] RS-I appears to assist the disposal of CO₂ given off by the cells, thereby maintaining the pH within safe levels for longer periods and effectively extending the dormancy periods. This dormancy offers flexibility, enabling sub-culture to be carried out at the researcher's convenience. Also, cells may be transported in this dormant state without freezing or using dry ice, thereby dramatically reducing logistics costs and facilitating transportation.

Tissues

AQIX[®] RS-I was developed to simulate the basic composition of the fluid environment surrounding every cell in all tissues and organs – the interstitial fluid (ISF) layer. This means that AQIX[®] RS-I is ideally suited to control the acid-base (pH) balance of tissues during storage, transportation or even perfusion at body temperature thereby enabling tissues to be used successfully for diagnostic and drug discovery purposes for up to 72 hours following removal. AQIX[®] RS-I has also been shown to retain the genetic and histological profiles of tissues for up to 72 hours.

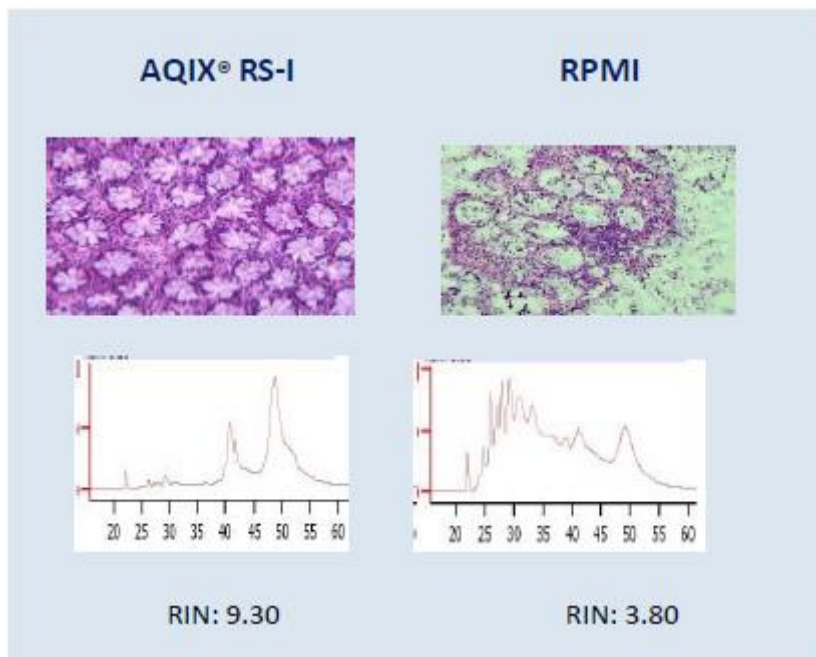
While other tissue preservation solutions are 'tissue-specific', AQIX[®] RS-I can be used with all types of normal and malignant tissues and is ideal for storing and transporting 'fresh' tissues for further processing.

AQIX[®]RS-I has been shown to be effective in many important preclinical research applications. The data below demonstrate how tissue samples can be transported in AQIX[®]RS-I prior to processing for histology or extraction of RNA.

Other proven applications include:

- Human cadaver organ reanimation
- Human stem cell isolation/Bioreactor incubation
- Drug Bioassay using Human intestine; colon; lung; atrial trabeculae preparations
- Normothermic perfusion using AQIX[®]RS-I + human RBC's

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Human colon biopsies were collected and transported for 30 hours at 4°C in either AQIX®RS-I or RPMI. Frozen sections were prepared and H&E stained and total RNA was extracted and analysed using the Agilent Bioanalyzer.

[Data courtesy of Cureline Biopathology LLC].

Procurement Management of Tissue Biopsy Samples

- Tissue biopsy samples are collected at procurement site as currently conducted
- Tissue samples are immediately placed into 125 mL of AQIX® RS-I for direct dispatch (25mL AQIX® RS-I per 1g tissue)
- Tissues may be further dissected into 3 sections whereby, one section is retained as a fresh sample, the second fixed in formalin with the third sample preserved under frozen conditions (see below)
- This procedure gives versatility in the final diagnostic analysis of tissue biopsy to cover all aspects of sample determinants in translational medicine

Comparison of Tissue Biopsy Procurement Methods

Collection stage	Current Practices	AQIX® RS-I Protocols
Collection and immediate holding	Biopsy delivered dry/soaked in saline/ wrapped in gauze/formalin fixed.	Biopsies simply immersed in requisite volume of AQIX® RS-I @ 3 - 8°C.
Transportation	Snap frozen (SF), Formalin fixed (FFPE) processing or sent as fresh	Fresh, FFPE and SF tissues can be maintained 'fresh' in AQIX® RS-I over ice @ 0 - 4 °C or room temperature

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	tissues. Long transport times may degrade fresh tissues.	(RT) for 12-72 hours before processing.
Processing	Fresh tissues may be utilised immediately otherwise availability is limited to SF or FFPE tissue samples	AQIX® RS-I allows for an unlimited choice of tissues and techniques – fresh tissues, FFPE and SF tissues.
Analyses	Analytical choices are limited owing to processing restrictions.	AQIX® RS-I allows for an unlimited choice of tissues and therefore analytical options.
Outcomes	Possibility for transformation of molecular profiles within tissue samples.	AQIX® RS-I retains the morphological and molecular structure of tissues including the functional activity.

Organs

The transportation and storage of organs has always been problematic due to time sensitive requirements in order to retain organ functionality. Current Donor organ preservation solutions are designed to preserve organs suspended in an inanimate, non-functional state under 'cold' conditions for limited time periods of 4 - 24 hours.

AQIX® RS-I was developed to be isosmotic and isoionic to serum and interstitial fluids, without the addition of colloids, common in many preservation solutions. Also, the superior pH buffering system based on the natural, α -stat pH buffering afforded by the imidazole groups within haemoglobin molecules makes AQIX® RS-I a superior alternative organ support medium for the support of organs under normothermic conditions. AQIX® RS-I has been used successfully in the following trials:

- I. The bodily functions of intravenously perfused organs of rat and pig models
- II. The functionality of ex vivo perfused rat, rabbit, guinea pig, pig and human tissues and organs
- III. Reanimation of cadaver, donor 'marginal' organs for use in bioassay investigations to evaluate new drug design
- IV. As a donor organ 'flush' fluid that facilitates better function following transplantation

Historically, donor graft function after transplantation has not been totally successful. The Consortium for Organ Preservation in Europe (COPE) has adopted AQIX® RS-I (GMP Grade) as the basic fluid composition in their quest to improve donor organ transplant technology in 2013-18.

Formulation

AQIX® RS-I contains only 15 perfectly balanced components to faithfully replicate the *in vivo* environment that naturally bathes all cells and tissues in the human body. Most other solutions used to collect human biospecimens incorrectly mimic the **intracellular** environment. AQIX® RS-I replicates the biophysical and biochemical characteristics of **extracellular**, ISF environment.

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- **pH Buffering System:** AQIX® RS-I uses a BES/HCO₃/pCO₂ to simulate Hb-imidazole pH buffering and contains no inorganic phosphate ion buffering, which is known to inhibit major metabolic and cell signalling pathways.
- **Ionic Composition:** Levels of Ca₂⁺, Mg₂⁺, Na⁺ and K⁺ in AQIX® RS-I are identical to the 'free' serum/ISF ionic levels
- **Metabolite & Energy Supply:** Essential components are provided in order to maintain metabolic homeostasis *per se*
- **AQIX® RS-I also contains the natural level of Human Insulin [28 mU/L]** which supports diverse cellular processes including glucose transport, protein, DNA and lipid synthesis

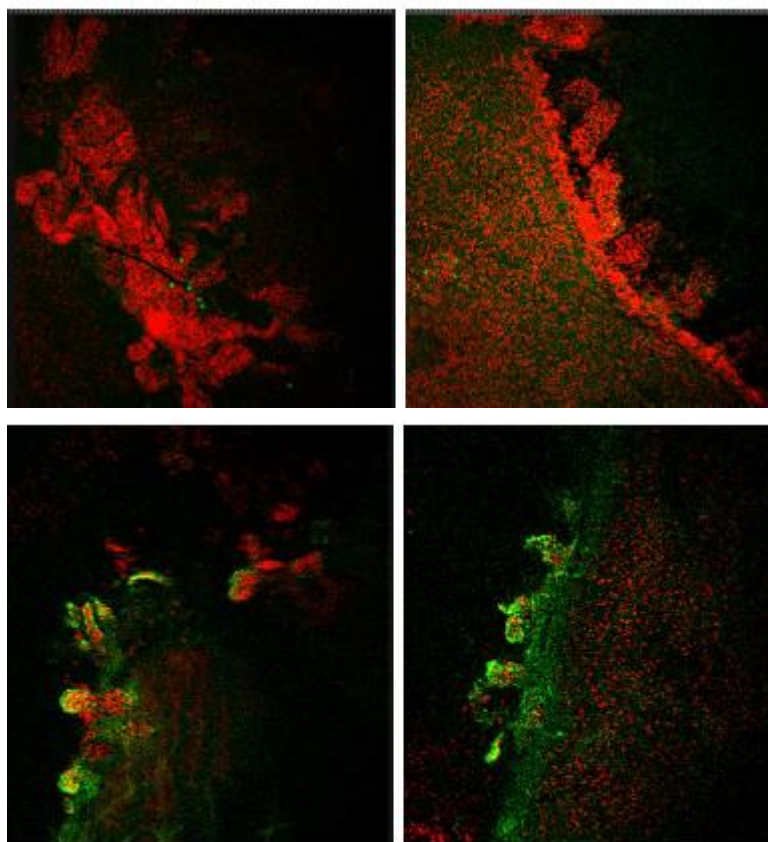
Using AQIX® RS-I

Tissues

Fresh human tissue should be placed directly into AQIX® RS-I immediately after removal from the donor and prior to storage, transportation or further processing.

Tissue samples have been shown to remain functionally viable and morphologically intact for up to 72 hours, the exact duration depending on the metabolic activity of the tissue and the temperature at which it is held. Preventing apoptosis and cell death is a major attribute accorded AQIX® RS-I by numerous investigators (see, below) in comparison to the use of current biopreservation media.

No further manipulation of the sterile RSI solution is required prior to use. Additional antibiotic preparations may be required depending on procurement conditions. Please contact us for further information.



Viability of Mouse Intestinal Biopsies after 24 Hour Storage on Ice [0-4°C]. [Staining: green=live; red=dead]

RPMI

AQIX® RS-I

Recommended AQIX® RS-I fluid volume requirements

Storage/Transit Time vs. Biopsy Size

Human Tissue Biopsy Type at 4 °C	Size (mm)	No. of Biopsy Samples Per Container	Required Vol. of AQIX RS-I (mL)	Transit time (hours)
Skin	10 x 10	5	250	1 – 12
Skin	10 x 10	1	125	20-36
Tonsils	10 x 15	1	125	20-36
Colon	10 x 20	1	125	37-48
Intestine	10 x 20	1	125	20-36
Lung: Bronchii (small)	10 x 12 diam x 5	1	125	13-19
Lung: Bronchii (large)	40 - 70 long	1	250	13-19
Heart: Atrial trabeculae	10 - 20 diam x 5	1	125	13-19
Heart: Atria/SA-node	20 x 40 long	1	125	13-19
Heart: P-M papillary m	30 - 60 long	1	250	13-19
Heart: Coronary arteries	10 - 50 long	1	125	13-19
Liver	15 x 15	1	125	20-36

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Pancreas	15 x 15	1	125	20-36
Breast: normal	10 x 10	1	125	37-48
Breast: malignant	10 x 10	1	125	37-48
Umbilical cord	50 long	1	125	73-96
Umbilical cord (@ RTP)	50 long	1	125	20-36

Cells

Storage / shipment* of adherent cells at ambient temperature using AQIX-RSI®

Prior to storage cells should be cultured under recommended conditions.

- At time of storage, aspirate the culture medium and gently wash cells with AQIX-RSI®, aspirate AQIX-RSI®.
- Add the same volume of AQIX-RSI® to the culture vessel that was being used to initially culture the cells i.e. if cells were cultured in 5 mL of complete medium in a T25 flask, 5 mL of AQIX-RSI® would be used.
- Ensure the culture vessel is sealed completely and store in the dark, at room temperature (16-25°C).
- At time of recovery, aspirate AQIX-RSI and replace with complete culture medium. Return the cells to their recommended culture conditions.
- Allow approximately 48 h for complete recovery, depending on cell-line.

* For shipment, the culture vessel should be filled completely with AQIX-RSI® and the vessel sealed tightly using parafilm, or equivalent.

Shelf Life

AQIX® RS-I medium has a shelf life of 8 months from the date of preparation when stored at +2 - 8°C under dark conditions.

Storage

Recommended storage is +3 - 8°C.

Protect AQIX® RS-I from exposure to light.

Shipping

Product ships at +4°C on cold packs.

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Awards

GHP International Magazine, Life Science Award 2016 “Best Human Biopsied Tissue Preservative Solution: AQIX® RS-I”.

Customer Testimonials

These case studies provide a short overview demonstrating the use of AQIX® RS-I medium in customer studies. Should you wish to view the full report, please contact us for further details.

Case Study: Phillips Group. Neural Tissue-engineering, University College London

Our group is interested in engineering nervous system tissue. As part of this, one of our projects is looking at developing therapies to repair the damaged spinal cord. Given that the mechanical properties of a tissue have the potential to profoundly alter cell behaviour, one aspect of this project has been to map the mechanical properties of spinal cord tissue to inform the design of future tissue-engineered constructs.

We are currently testing rat spinal cord tissue with dynamic mechanical analysis (DMA). In order to keep spinal cord tissue as comparable to *in vivo* as possible, we initially used phosphate buffered saline (PBS) as a transport media prior to mechanical testing. However, in an effort to minimise any mechanical changes which may occur temporally post-mortem as a result of cell death, we have now refined our protocol to include AQIX RS-I as the media of choice.

Case Study: Parsortix Ltd.

Efficacy of AQIX® RS-I versus HypoThermasol solution to isolate circulating tumour cells using a Parsortix cell separation system

AQIX® RS-I versus BioLife’s HypoThermasol solution were tested at both 4°C and RTP to isolate A549 and SKBR3 tumour cells over a time course of up to 72 hours.

Results:

AQIX® RS-I was superior to BioLife’s HypoThermasol solution at 4°C and also at RTP for both A549 and SKBR3 cell lines.

AQIX® RS-I demonstrated greater superiority than BioLife’s HypoThermasol solution at 4°C.

Case Study:

Use of AQIX® RS-I in Petaka-G3 Cell Culture Device

The Petaka-G3 module allow cells to be grown in their normal physiological conditions with normal ambient CO₂, O₂ & humidity levels. This a perfect tool for stem cell & primary cell cultures. The Petaka-

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G3 module also offer one other unique application. At 75% confluence, one can induce the cells into a state of lethargy called dormancy or suspended animation, by simply withdrawing the culture from heat & keeping it in a hypothermic condition between 16-21°C. Cells in this state may be kept up to 60 days without media change though there are quite big variations according to the cell line. Dormancy may be extended with media changes.

We have done some preliminary work using AQIX® RS-I. We have observed that the addition of AQIX® RS-I does give a boost speed of cell division which is especially interesting for cells with a higher respiration rate such as HEPG2 or monoclonal antibodies. In addition to giving a boost to the culture, the AQIX® RS-I seems to provide a means of disposing of the CO2 given off by the cells thus keeping the pH within safe levels for longer periods, thus effectively possibly extending the dormancy periods. The dormancy does offer infinite flexibility in the lab enabling sub-culture to be done at the researcher's convenience. Also, cells may be transported in this state without freezing or using dry ice thus dramatically reducing logistics costs & making the receipt of cells less of an operation of urgency than in the current common methods.

Case Study:

Use of AQIX® RS-I in the Acea xCelligence System

We had outstanding results using AQIX® RS-1 solution as an alternate buffer to HBSS in the Acea xCelligence system.

We saw very clean and stable signals and tight CVs looking at GPCR receptor signalling. The AQIX® RS-I kept the cells extremely happy and we were able, after one GPCR response died down, to re-stimulate and so on, over several hours. We saw this with 5 of 5 different cell lines and depending on the experimental design, detected GPCR agonist and antagonist activities.

I recognize that AQIX® RS-I does not have pH dye indicators but there was no problem with pH stability in assays conducted over several hours because of the robust buffering you use.

The AQIX® RS-I system was so sensitive we were even able to record the relatively weak and transient (<2mins) signals from a kinase receptor ligand, suggesting to me that use of AQIX® RS-I could further extend the 'sensitivity' of the Acea xCelligence System analyses.

Case Study: Biostor Ireland, Ion Channel Biotechnology Centre

Rabbit Bladder Smooth Muscle Cell Study

In our laboratory we routinely disperse fresh smooth muscle cells from the rabbit bladder on a daily basis as we have noticed that they begin to lose function over 24 hours following dispersal.

Indeed this is what we observed in this study, in our control group, on day 2 cells had already lost the ability to release calcium from intracellular stores in response to caffeine and by day 3 they had lost all contractile functionality. AQIX preserved function in bladder SMC up to and including day 4.

Case Study: Wittenburg Group. Tissue Engineering Department, Clinic for Oral- and Maxillofacial Surgery, University Hospital, Carl Gustav Carus Dresden

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AQIX® RS-I was tested in comparison to three commercial media A-C on adherent cells (monolayer of either human gingival fibroblasts, keratinocytes or endothelial cells), on cells seeded onto a collagen matrix (single constructs with either human gingival fibroblasts, keratinocytes or endothelial cells) and on collagen matrices, which were seeded with all three types of cells at once (triple constructs).

AQIX® RS-I is completely suitable as a transportation medium either for adherent cells (hGF, NHEK), matrix-bound cells (hGF, NHEK) or tissue engineered cell constructs (made of hGF, NHEK and HUVEC). Cell survival and proliferation can be guaranteed exclusively for 24h. Expanded incubation times 48-96h cells do not show any signs of vitality.

In Summary, hGF, HUVEC and NHEK as monolayers survived and proliferated well in AQIX® RS-I or Standard medium, but when seeded on collagen scaffolds all three cell types showed less vitality parameters. Triple constructs incubated for 3 days under standard conditions and afterwards for 24h at RT w/o CO₂ in AQIX® RS-I showed a counterbalanced vitality and performed better in AQIX® RS-I than in Standard medium. Morphological changes observed by light microscopy advise the use of AQIX® RS-I for not longer than 24 hours.

AQIX® RS-I is completely suitable to produce cryopreservation medium with 5% DMSO for suspension and matrix-bound cells (hGF). Cell survival and proliferation can be guaranteed for **one** week.

Case Study:

Advancement in Preserving Human Tissue Morphology and Gene Expression Profiling: Human Colon Tissue Biopsies

Tissue samples stored and transported in AQIX® RS-I solution showed superior preservation of both morphology and RNA integrity in comparison to those tissue samples stored and transported in RPMI solution.

We have shown that if fresh human tissue biopsies are immersed immediately in AQIX® RS-I, then the cells comprising these biopsied tissues maintain a homeostatic balance during transportation under hypothermic conditions.

The results indicate that AQIX® RS-I solution significantly advances the ability to store and transport cancerous tissue samples over periods of time in a condition that will preserve their morphology, RNA integrity and thereby facilitate more accurate expression of gene profiles to advance diagnostic and prognostic outcomes.

References:

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Further Reading

Additional technical information is available on request, including Applications and SOPs:

1. Application: Use of AQIX® RS-I for lymphocyte culture.
2. Application: Platicell Ltd. Comparison of the effects on functionality and cell differentiation of Mouse ES-derived cardiomyocytes following incubation in conventional TC-media versus AQIX® RS-I solution.
3. Application: Cureline. Use in Fresh Human Tissues and Blood samples.
4. Application: Procurement Technology for Human Tissues.
5. Technical Summary: Biopta Ltd. Preservation and Transport of *Ex Vivo* Human Cardiac Tissue for *In Vitro* Pharmacology Studies.
6. Technical information: formulation details
7. Method: Using AQIX® RS-I (10X) and (1X) RTU solutions
8. Method: G-I Tract Tissue Biopsy Procedures. Storage, Retrieval and Transport of Rat small intestine/colon biopsies.
9. Method: Perfused Human Colon Biopsy Procedures. Storage, Retrieval and Transport of biopsies.
10. Method: Equine Umbilical Cord Collection.
11. Method: Human Colon Biopsy Procedures. Storage, Retrieval and Transport of biopsies.
12. Method: Intestine Tissue Biopsy Procedures. Storage, Retrieval and Transport of biopsies.
13. Method: Human Atrial Appendage Biopsy Procedures. Storage, Retrieval and Transport of biopsies.
14. Method: Human Skin Biopsy Procedures. Storage, Retrieval and Transport of biopsies.
15. Method: Human Bronchi Biopsy Procedures. Storage, Retrieval and Transport of biopsies.
16. Method: USSING Intestine Biopsy Procedures. Storage, Retrieval and Transport of biopsies.
17. Method: Human Umbilical Cord Collection Protocols. Storage, Retrieval and Transport of biopsies.
18. Method: Human Liver Biopsy Procedures. Storage, Procurement and Transport of biopsies.
19. Method: Blood Dilution Procedures.
20. Method: Hepatocyte Protocols.
21. Method: PBMC Isolation Protocol.

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22. Method: Application of Carbogen Gas.
23. Method: Cryopreservation of HS-cells.
24. Method: Human Myometrial Cell Isolation.
25. Method: Isolation of CD34+ Cells from Whole Blood.
26. Method: Procurement of Cadaver Human Hearts and Component Parts of the Anatomy.
27. Method: Human Pancreatic Biospecimen Procurement. Long term [24-72 hours] procurement of pancreatic tissue biopsies.
28. Method: Human Skin Biospecimen Procurement. Procurement of tissue biopsies.

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Support

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