RDI Hepatocyte Retrieval Protocols

A. In Laboratory:

1. Place approximately **800 cm^3** of MilliQ® water in a **1 L** volumetric flask and warm to 32 - 36 EC while aerating with carbogen [95% CO_2 / 5% O_2] for 15 - 20 minutes.
1. Place a funnel into the 1L flask and add **2.1 g** of Sodium bicarbonate and rinse into the flask with MilliQ® water
2. Now carefully pour (via the funnel) the contents of the 10x concentrate of bottle of AQIX® RS-I solution .
3. Rinse the AQIX® RS-I solution bottle TWICE into the funnel and then add MilliQ® water to the graduated 1L mark.
4. Shake the 1L flask and record pH of 1x solution of AQIX® RS-I **@** 32 - 36 °C
5. Vacuum fill a 1L Infusion Drip bag and place in a sealed container to retain temperature during transportation to liver organ retrieval site.

B. **At Organ Retrieval site:**

5. Infuse AQIX® RS-I solution at 32 - 36 °C temperature into the donor liver organ or lobe adopting a double perfusion procedure, namely via **hepatic portal vein** and also simultaneously (suggestion) via the **hepatic artery** (?)
6. This procedure should take about 5 minutes and use about **200-500 cm^3** of AQIX® RS-I solution
7. Transfer perfused donor liver organ/lobe back to the laboratory totally immersed in AQIX® RS-I solution within a sealed plastic bag over wet ICE [4 - 8 °C]
8. The perfused organ can now be (a) stored overnight in a fridge [8-12 °C] or,
   (b) dissected and the hepatocytes prepared as below (9)
5. **Isolate** and then **store** hepatocytes from the liver organs retrieved by transfer procedures (a) and (b) in the following manner;
   (i) AQIX® RS-I solution @ 0 - 4 °C
   (ii) AQIX® RS-I solution @ 8 - 12 °C
   (iii) AQIX® RS-I solution @ ***ambient*** temperatures
   and, for comparison;
   (i) UW solution @ 0 - 4 °C
   (ii) UW solution @ ***ambient*** temperatures

10 Assess % viability of hepatocytes [Trypan Blue dye exclusion, P450, cholesterol synthesis] after storage under conditions (i) - (v) for;
   (A) 24 hours (B) 48 hours

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