[SOP/H-UC/ST/11-08]

Human Umbilical Cord Collection Protocols:

Method:

Step 1. – Umbilical Cord Procurement Site Preparation

Prepare a clean, sterile surface area in the ante-room to the operating theatre.

Remove the bottle of AQIX® UC fluid and swab the outside of the bottle with a suitable sterilising agent. Assemble the bottle of AQIX® UC fluid along with surgical gloves, face mask, scissors and forceps on the prepared surface.

Step 2. – Collecting the Umbilical Cord

Sample Retrieval Procedures:

a) Put on surgical gloves and face mask and retrieve the placenta plus umbilical cord biopsy from operating theatre and transfer to the prepared area selected.

b) Using the sharp scissors, carefully cut the cord about 5-10cm from the placenta.

c) Holding the umbilical cord (UC), cut through the remaining placenta leaving a 5 - 10cm) circular rim of the outer, epithelial (amnion) layer containing multipotent cell types (Miki et al, 2005).

d) Using forceps, mechanically peel off the chorion from the amnion layer to form the UC-amnion sample.

e) Thoroughly rinse the 'UC- amnion' biopsy several times using AQIX® UC-fluid and finally store/transport the UC-amnion HSC sample in a 250 mL bottle of AQIX® RS-I **or** its antibiotic (RS-I/Ab) **or** antifungal (RS-I/Af) **or** combination (RS-I/Ab+Af) formulations.

f) Screw lid firmly onto the UC-sample bottle and ensure that there are no leakages.

g) Place AQIX® UC-sample bottle into Transport Box for dispatch to a laboratory for further processing.

Step 3 – Storage & Transportation Procedures:

A) Hypothermic Conditions (recommended)

l) Place a ***frozen*** cool pack on the bottom of the polystyrene transportation box and the other ***'cold'*** but ***unfrozen*** cool pack on top of the solid, ***frozen*** cool pack to act as a 'cushion' for the AQIX® UC-sample bottle.

m) Place the AQIX® UC-sample bottle on top of the ***unfrozen*** cool pack and fill the surrounding spaces with sterile packaging to stop the sample bottle moving about and to retain a low temperature during transport.

n) Place the lid on the transportation container and tape closed the cardboard sleeve to ensure contents are safe.

o) Dispatch transportation to recipient laboratory for isolation of placenta (amnion) and chord HSCs.

A) Ambient Conditions

l) Place the AQIX® UC-sample bottle into a polystyrene transportation box and fill the surrounding spaces with sterile packaging to stop the sample bottle moving about and to retain a low temperature during transport.

n) Place the lid on the transportation container and tape closed the cardboard sleeve to ensure contents are safe.

o) Dispatch transportation to recipient laboratory for isolation of placenta (amnion) and chord HSCs.

Step 4 – Isolation of Umbilical Cord (UC) and Amnion Epithelium (AE) Derived Cells:

p) Remove the sample from transportation container and;

1.	Either,	Prepare UC-tissue for immediate extraction and culture of stem cells as described by Miki et al., 2005 [Stem Cells.2005;23(10):1549-59]
	Or,	
2		Subdivide UC-tissue for short-term preservation (1 - 5 days) in 'fresh' AQIX RS-I @ 3-8 °C
	Or,	
3		Freeze gradually over 16-24 hours @ **-60** °C for Long-term storage using a suitable cryopreservation solution (DMSO; AQIX RS-I + 5% Glycerol)

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