AQIX® SOP – Human Myometrial Cell Isolation

Preparation of Human Myometrial Tissue Biopsies and Cells

Procurement: Human myometrial biospecimen samples [10 mm x 10 mm] are biopsied from 'late-term' pregnant donors and immediately placed into a 125 mL AQIX® RS-I kit (8 per kit) and maintained over ice during storage/ transportation for the further isolation and preparation of immortalized PHM1-41 cells.

Solutions:

Storage/Transportation and Perfusion solution

- AQIX® RS-I (1x) [can contain 1x Streptomycin/Penicillin (Sigma-Aldrich, P4333)]

AQIX® Collagenase solution (1.5 mL aliquots)

- 1.5 mL of AQIX® RS-I (includes serum levels of calcium and magnesium ions)
- 15 µL of 2000 U/mL DNase I (Sigma-Aldrich, DN25; stock solution: 2000U/mL in DNase buffer [10 mM tris-HCl, 50 mM NaCl, 10 mM MgCL2, 1 mM DTT, 50% glycerol, pH 7.5])
- 15 µL of 100x Streptomycin/Penicillin (Sigma-Aldrich, P4333)
- 130 µL of 12500 U/mL collagenase (Sigma-Aldrich, Collagenase Crude: Type IA, C2674; stock solution: 12500U/mL in AQIX® RS-I). **Note:** the collagenase is added just prior utilization.

AQIX® Trypsin-EDTA solution (1.5 mL aliquots)

- 1.5 mL of AQIX® RS-I (includes serum levels of calcium and magnesium ions)
- 130 µL of 10x trypsin-EDTA (Sigma-Aldrich, 59418C)
- 15 µL of 100x Streptomycin/Penicillin (Sigma-Aldrich, P4333)

AQIX® RS-I 'Wash' solution (1.5 mL aliquots)

- 1.5 mL of AQIX® RS-I
- 15 µL of 100x Streptomycin/Penicillin (Sigma-Aldrich, P4333)

Formulation of AQIX® MM culture medium (50 mL) [OPTIONAL] or use DMEM

- 16 µg/mL putrescine (Sigma-Aldrich, P6024; stock solution – 161.1 µg/mL in AQIX® RS-I medium)
- 0.0063 µg/mL Progesterone (Sigma-Aldrich, P6149; Stock solution 20 µg/mL in AQIX® RS-I medium with 2% EtOH)

- 0.0052 µg/mL sodium selenite (Sigma-Aldrich, S5261; Stock solution 20 µg/mL in AQIX® RS-I medium)
- 100 µg/mL transferrin (Sigma-Aldrich, T8158; stock solution 10 mg/mL in AQIX® RS-I medium)
- 2% (v/v) B27 Supplement (Gibco, B-27 50x, 12587-010)
- 1 mM LiCl (Sigma-Aldrich, 8M solution, L7026)
- 10 µM ATP (Sigma-Aldrich, Adenosine 5'-triphosphate disodium salt, A6419; stock solution 36,3 mM (20 mg/mL) in sterile H2O)
- 2 µg/mL Heparin (Sigma-Aldrich, H3149; stock solution 10 mg/mL in sterile H2O)
- 0,72 µg/mL Prolactin (Sigma-Aldrich, L4021; stock solution 10 µg/mL in AQIX® RS-I + 0.1 % BSA)
- 20 ng/mL EGF (Gibco, PHG0311; stock solution 2 µg/mL in AQIX® RS-I + 0.1 % BSA)
- 20 ng/mL FGF (Gibco, PHG0024; stock solution 2 µg/mL in AQIX® RS-I + 0.1 % BSA)
- 10 ng/mL LiF (Sigma-Aldrich,L5283; stock solution 1 µg/mL in AQIX® RS-I + 0.1 % BSA)
- 2.5 µg/mL Amphotericin B (Sigma-Aldrich, A2942)
- 1 x Streptomycin-Penicillin (Sigma-Aldrich, P4333)
  [Note: all the prepared stock solutions from powders are filter-sterilized after reconstitution in sterile, filtered AQIX® RS-I medium prior to use]

Myometrial Tissue & Cell Preparation Procedures:

Upon arrival in the laboratory, each 10 mm x 10 mm biopsied tissue sample is placed into a 15 mL polypropylene tube containing, 5 mL of 'fresh' AQIX® RS-I medium plus (1x) Streptomycin/Penicillin.

The following procedures are then performed in a sterile environment (Laminar Flow Hood):

1. Transfer the biospecimens to a tissue culture disk containing fresh, AQIX® RS-I medium, and cut each tissue sample into smaller pieces (approximately 1 - 3 mm^3) using a sterile scalpel blade.

2. Transfer the dissected tissue blocks and medium into a 15 mL polypropylene tube.

3. Centrifuge for 5 minutes at 800xg.

4. Remove the supernatant and add 1mL of the AQIX® collagenase solution.

5. Place the tube in a water bath at 37ºC with agitation for 60 minutes and apply mechanical digestion (up and down with a micropipette yellow tip) every 20 minutes. Note: At the end of the first 20 minutes, transfer the mixture into two, 1.5 mL microcentrifuge tubes to accentuate mechanical digestion for the remainder of the 60 minutes.

6. At the end of the 60 minutes, centrifuge the two microcentrifuge tubes for 5 minutes at 800xg and remove the supernatant solutions.

7. Add 700 µL of the AQIX® trypsin-EDTA solution into each microcentrifuge tube.

8. Place the microcentrifuge tubes into a water bath at 37 ºC with agitation for 5 minutes to facilitate mechanical digestion. Finally, add 700 µL of AQIX® RS-I medium to inactivate trypsin.

9. Centrifuge for 5 minutes at 800xg and remove supernatant solution.

10. Again 'wash' with 1mL of AQIX® RS-I medium and centrifuge for 5 minutes at 800xg and remove supernatant solution.

11. Repeat the 'wash' step twice.

12. Re-suspend the cells in each tube with 1 ml of the AQIX® MM culture medium.

13. Plate 500 µL of the cellular suspensions into each well of a 24 multi-well plate and then add 1 mL of the AQIX® MM medium to each well containing PHM1-41 cells.

Transfection & Electroporation procedures

Proceed as directed in Section2.4 but 'wash' the PHM1-41 & UtSMC cells with AQIX® RS-I **not PBS!**

Transfect and Electroporate as previously described (2.4) but dilute into 500 µL of pre-warmed AQIX® RS-I medium.

Dispense cells as previously described but using AQIX® RS-I medium for Ca^{2+} - imaging [80 µL] and RNA analysis [350 µL] , incubating for 24 hours in an humidified 5% CO_2 / air mixture.

Renew AQIX® RS-I medium every 24 hours and analyze cell samples at 48, 72 and 96 hours following electroporation to assess % efficiency.

Measurement of $[Ca^{2+}]_i$ (Section 2.7)

Make up 5µM Fura-2-AM and 0.1% Pluronic F-127 AQIX® RS-I medium (pH 7.25 -7.46; **do not add any acid/alkali as AQIX RS-I solution pH is auto-regulated over physiological temperatures!)**

Proceed as directed in Section 2.7 but using AQIX® RS-I medium only as the 'buffer'.

Electrophysiological Measurements (Section 2.8)

Simply use AQIX® RS-I medium + 100 nmol/L nifedipine as the bath perfusate in the recording chamber as it has been used similarly to record miniature endplate potentials from *in vitro* maintained human intercostal muscle fibres over 20-30 hours (Rees, 1978).