Washing Tubing Instructions

Introduction

These instructions are intended to guide the embryologists (working at the IVF laboratory) on how to prepare a biopsied sample to be sent for testing at Igenomix UK. This procedure can only be performed by embryologists that have demonstrated competence in biopsy/tubing.

Note: The embryologist should follow the standard operating procedures set by the IVF clinic for embryo biopsy.

Materials

- Laminar Flow Hood with UV lamp.
- Fine or superfine permanent marker.
- Stereomicroscope/inverted microscope for cell micromanipulation.
- Single channel pipette 1 µl- 10 µl (used exclusively for this procedure and always handled with gloves).
- Filtered Sterile tips (DNA/RNA free). Kept in sterile conditions.
- Petri dishes (60 mm). Kept in sterile conditions.
- Micromanipulation pipette/capillary (i.e. Vitrolife Denudation Pipette (122-124µm) for blastocyst biopsy or Origio Denuding Pipette (75µm) ref MXL3- 75 for blastomere biopsy.
- Micromanipulation pipette/capillary holder or Stripper (i.e. from Vitrolife or Origio).
- Mineral oil.
- 70% ethanol or DNA AWAY™ Surface Decontaminant.
- (Not mandatory) - minicentrifuge for 0.2ml microcentrifuge tubes (i.e. VWR MiniStar silverline cat#521-2845)
- Kim wipes.
- One "Tubing kit" provided by Igenomix UK that contains the following:
    a) "Plate/rack" holding sterile 0.2ml microcentrifuge (PCR) tubes labelled by a unique ID number (side wall).
    b) Sterile 1.5 ml tube containing 1.5 ml of cell "washing/tubing" buffer (1xPBS/0.1%BSA).
        a) & b) are placed together in a sterile plastic bag that must remain sealed until the biopsy.
    c) Ice packs. Once the kit is received, the ice packs should be placed in the freezer (-20ºC or -80ºC). These ice packs will be used to keep the samples "chilled" once shipping back to Igenomix UK.
    d) Cooler where a), b), and c) are placed inside prior to shipping.

General instructions to prevent contamination

- Use sterile gloves for handling all materials of this procedure.
- Wear a disposable lab gown, cap and mask.
- Prior to conducting this procedure clean with a DNA degrading solution (using a Kim wipe) the internal/external surface of the Laminar Flow Hood and the external surface of all materials used

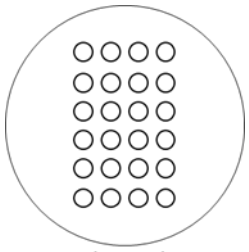
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(stereomicroscope, Petri dishes, tip boxes, single channel pipette, boxes containing Micromanipulation pipettes/capillaries, micromanipulation pipette/capillary holder, minicentrifuge).
Place the sterile material inside the Laminar Flow Hood and switch on the UV lamp for a minimum of 15 minutes. The "tubing" must be performed inside the Laminar Flow Hood and under the stereomicroscope.

Protocol

A "witness" is highly recommended for this procedure. It is recommended to keep the plate holding the 0.2ml microcentrifuge tubes on ice or use a pre-chilled PCR plate.

- Use a new capillary for each individual biopsied sample (i.e. trophectoderm sample).
- "Washing/Tubing": Use a single channel pipette to prepare a Petri dish (ideally 60 mm) according to the diagram shown below with a maximum of six rows comprised of four droplets of 5µl each of "washing/tubing" buffer. Cover the setup with 8 ml of mineral oil at room temperature. Use one row per biopsied sample. Each sample must be rinsed in the first three droplets of a row. Take the sample along with a 1ul-2ul "washing/tubing" buffer from the third droplet and load it into a 0.2ml microcentrifuge tube (the sample must be observed entering in the 0.2ml microcentrifuge tube by the embryologist conducting the tubing). The fourth droplet can be used for checking the capillary in case the sample is not seen entering the 0.2ml microcentrifuge tube. In case the sample appears in the fourth droplet, discard the 0.2ml microcentrifuge tube where the sample was supposed to be loaded and load the cell into a new tube.



Washing plate

- Creating "Blanks": (Required only for the "embryologist validation" and rare cases done by conventional PGT-M which is an outsourced test). After releasing each sample into the 0.2ml microcentrifuge tube aspirate a small volume (1ul-2ul) of the last "washing/tubing" drop (usually droplet #3) with the same capillary and release into a new 0.2ml microcentrifuge tube that will be designated as the "Blank" for that sample.
- Close the 0.2ml microcentrifuge tube, label the lid with the female patient initials followed by the embryo number using a fine or superfine permanent marker.
- Place the 0.2ml microcentrifuge tube in the "plate/rack".
- Record on the "PGT Test Requisition Form & Biopsy Worksheet" the patient initials, embryo number and the tube's unique ID number (provided by Igenomix UK and on the side wall of the tube) linked to it.
- Label the lid of the "plate/rack" with the patient name, patient date of birth and the unique patient ID number.
- Slip the "plate/rack" inside the provided sterile plastic bag and place in the cooler sandwiched between ice packs (retrieved from the -20ºC or -80 ºC freezer).

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- Include the completed “PGT Test Requisition Form & Biopsy Worksheet” (placed in a separate sleeve) in the cooler.
- Arrange for your own courier or contact Igenomix UK to arrange for transportation.

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