



# Single cell washing and handling

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## Washing and loading of single cells into PCR tubes

This document describes the steps involved in washing single cells (polar body 1, polar body 2, blastomeres and trophectoderm biopsy) that have been dissected from an oocyte, embryo or blastocyst and loading them into PCR tubes for use with SurePlex and subsequently 24sure and 24sure+ technologies.

The information supplied in this document is offered as a guide only and BlueGnome accepts no responsibility for carrying out the procedure. Good laboratory practice and good scientific judgement are required at all times. The protocol may need adaptation according to local laboratory rules, regulations and the facilities available.

Appropriate witnessing may be required and proper procedures should be established in the local setting.

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## Precautions

DNA contamination into the PCR tubes must be minimised at all times. Extraneous, contaminating DNA could result in an incorrect diagnosis and lead to a serious adverse outcome.

DNA contamination can be minimised by:

- Keeping gloves sterile and clean at all times.
- Changing gloves immediately you suspect contamination has occurred.
- Handling PCR tubes carefully, as described, only touching the outside.
- Never touching the 'dirty' end of the mouthpipette i.e. the mouthpiece and proximal tubing.
- Never allowing the tubing of the mouth pipette to touch skin.
- Carrying out the procedure, including the setting up of culture dishes and PCR tubes, in a laminar flow hood.
- Avoid keeping PCR tubes open for longer than necessary.
- Wear appropriate personal protective equipment (lab coat, gloves, cap, facemask).

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## Equipment

**Sterile PBS** (Cell Signaling Technologies 9808), diluted 1x with sterile nuclease free water and aliquoted into 1 ml volumes. Aliquots can be stored at -20°C.

**Sterile 0.2 ml PCR tubes** stored in small sterile bags.

**Culture medium** containing 4mg/ml albumin

**Sterile, long Pasteur pipettes**, pulled and flame polished (one for each cell plus a few extra)

**Mouth pipette** - using a mouth pipette is highly recommended for loading very small volumes with the cell – ultrafine control can only be achieved with a mouth pipette

**35 mm sterile culture dishes**

**0.2 ml PCR tube rack**

**Chilled 0.2 ml PCR tube rack**

**Dissecting microscope**

**Sterile forceps**

**Dedicated 10 µl pipette**

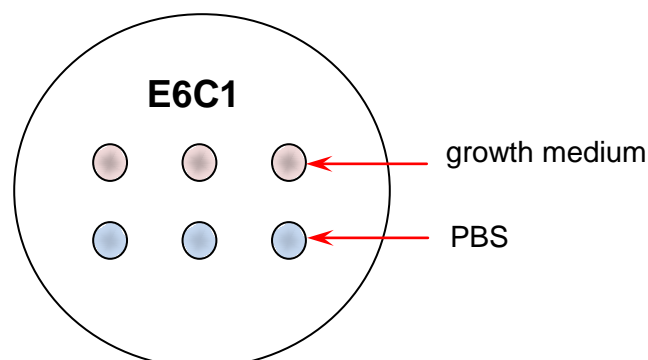
**Sterile 10 µl filter tips**

**Fine tipped permanent marker pen**

## 1. Preparation

To perform this procedure it is necessary to wear clean theatre dress and a long sleeved gown over the top. Sterile gloves must be worn, covering the cuffs of the gown, and hair must be covered with a cap. The preparation of dishes and tubes must be carried out in a clean laminar flow hood to protect the tubes from contamination.

- 1.1 In the hood remove enough culture dishes from the sterile packet as there are cells, so one dish is suitable for one cell.
- 1.2 Label the dishes appropriately in accordance with local procedures on both the lid and the base. For example with the embryo number and cell number i.e.E6C1 would be cell 1 of embryo number 6.
- 1.3 Using a clean, sterile Pasteur pipette add 3 small drops (~15-20 µl) of growth medium and three small drops (~15-20 µl) of PBS arranged as shown below:



- 1.4 Once the dishes are prepared leave to one side, within reach of the microscope.
- 1.5 In the hood, open the sterile autoclave bag containing the PCR tubes. Remove the tubes one by one from the bag, using the clean forceps, and place them in the tube rack (not the cold one). Close the lids immediately taking care not touch the inside of the lid.
- 1.6 Each cell requires one 0.2 ml sterile tube. Using a fine tipped marker pen, label the tubes in a similar way to the dishes, i.e. E6C1. For each patient it is necessary to collect a small volume of PBS from one of the last cell washes to provide a negative control in the SurePlex reaction.

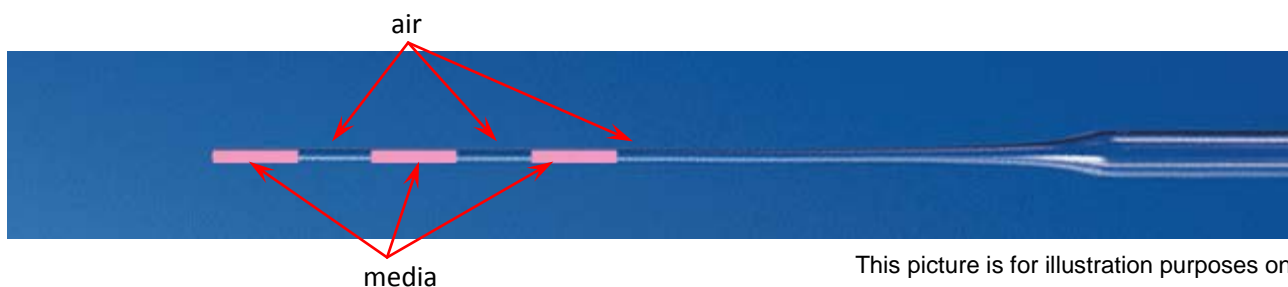
It is necessary therefore to prepare one extra tube per patient and label accordingly. Tubes should be labelled on the top and side.



- 1.7 Using an aliquot of PBS add 2.0  $\mu$ l of PBS to each of the tubes with the 10  $\mu$ l pipette and sterile filter tip. Be extremely careful not to touch the tip anywhere other than inside the tube. Change to a clean sterile tip every 3 or 4 tubes or whenever contamination is suspected.
- 1.8 Once all the tubes are prepared, place in a rack in the appropriate order that they will be required.

## 2. Cell wash protocol

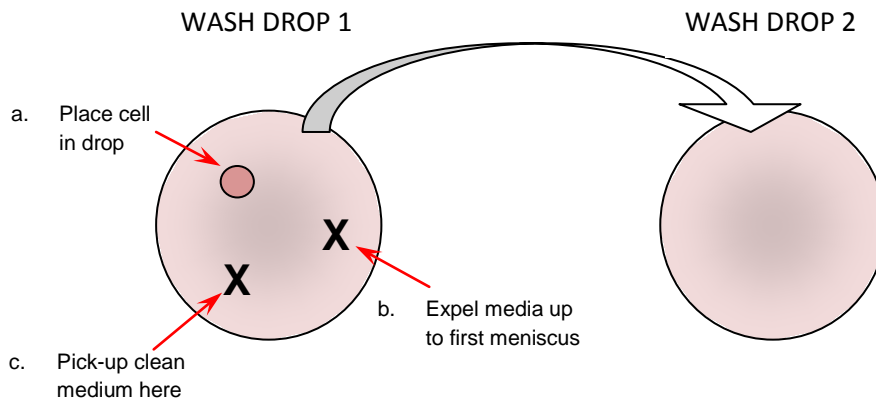
- 2.1 Put the mouth piece of the mouth pipette in the mouth, being extremely careful not to allow gloves to touch the mouth or skin.
- 2.2 Place the dish containing biopsied cells on to the microscope stage. This is a likely witnessing step to ensure that the patient ID and the embryo number on the dish containing the cells matches the labelling on the culture dishes and PCR tubes.
- 2.3 Put a pulled, flame-polished Pasteur pipette into the mouth pipette. Load the pipette with clean culture medium (plus albumin) so that there are three sections of medium separated by air, see below.



This picture is for illustration purposes only

- 2.4 Looking down the microscope, pick up a cell from the dish on the microscope stage in a minimal volume of media and place in the top left droplet (wash drop 1) of medium in the labelled wash dish (a) (shown overleaf).
- 2.5 Move the pipette to another location in that same drop and expel any remaining media up to the first meniscus of the pipette (b). Move the pipette to another location still in the same drop

and pick up a small volume of media (c). Pick up the cell in minimal volume and move it to the second drop (wash drop 2) of the same dish.



- 2.6 Repeat a-c in wash drop 2 and 3, and through the PBS wash drops, 4 – 6.
- 2.7 At wash drop 6, pick up the cell in a small volume of PBS, making sure the cell is not too close to the tip of the pipette or too close to the meniscus. Whilst holding the pipette, pick up the correctly labelled PCR tube, open the lid using the lip only and hold the tube sideways on the stage of the microscope so the PBS can be visualised at the bottom of the tube.
- 2.8 Observe the pipette tip by eye as you carefully insert it into the tube. Great care must be taken to avoid the pipette touching the outside of the PCR tube, and to avoid breaking the pipette into the tube.
- 2.9 Use the microscope to observe the pipette tip as it goes into the PBS, trying to avoid touching the pipette on the side of the tube.
- 2.10 Gently expel the cell into the PBS in a minimal volume which should be less than 0.2 $\mu$ l. Remove the pipette from the tube, close the lid of the tube and place it in the chilled PCR tube block.
- 2.11 To collect PBS wash for use as a negative control, use the same pipette, go back to wash drop 6 and pick up a small volume of PBS (the equivalent to that transferred with the cell) and carefully add it to the appropriate PCR wash tube.
- 2.12 Discard the pipette. If the cells are going to be processed within 24 hours store at 4 °C until used, alternatively freeze, whilst retaining the PBS, including cells, at the bottom of the tubes.

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