

CombiPGS™ and CombiPGD™ Packaging & Post Biopsy Cell Handling Guidelines

This document will guide you through the necessary steps involved from the receipt of the CombiMatrix PGS shipper through the return of the sample:

1. Storing the PGS Shipper
2. Precautionary measures to minimize DNA contamination
3. Cell Wash Preparation & Materials Required
4. Loading Protocol For Blastomere and Trophectoderm Cells
5. Return Shipping Instructions
6. Ordering additional PGS sample collection kits and shippers

1. How To Properly Store Your PGS Shipper With Cooling Packs

Upon receipt of your CombiMatrix Preimplantation Genetic Testing Styrofoam Shipper please:

- 1.1 Remove the 5 ice packs and place them flat in your laboratory freezer. Maintain the freezer temperature below -10°C (14°F) in order for the ice packs to achieve a solid frozen state.
- 1.2 Allow for a minimum of 24hrs for the ice packs to become **frozen solid; this is critical** in order for them to keep the biopsied cells frozen during the shipping process.
- 1.3 Confirm that the ice packs are in a solid frozen state prior to shipping. You should not be able to detect any movement within each of the icepacks; if you do, they are not ready to be used.

2. Precautionary Measures For DNA Contamination Prevention

Extraneous DNA could result in an incorrect diagnosis and lead to a serious adverse outcome. Please consider the following preventive measures to minimize DNA contamination:

- 2.1 Keep gloves sterile and clean at all times.
- 2.2 Change gloves immediately if you suspect contamination might have occurred.
- 2.3 Handle PCR tubes carefully and only touch the outer surfaces.
- 2.4 Carry out the procedure, in a clean laminar flow hood.
- 2.5 Avoid keeping PCR tubes open for longer than necessary.
- 2.6 Wear appropriate personal protective equipment (lab coat, gloves, cap, and facemask).

3. Cell Wash & Loading Preparation

The preparation of dishes and tubes must be carried out in a clean laminar flow hood using sterile gloves to minimize contamination.

- 3.1 Keep the PCR tubes cold during the loading process by placing an ice pack underneath them.
- 3.2 Remove the provided wash buffer provided by CombiMatrix from the freezer and let it warm to room temperature.

- 3.3 Each CombiMatrix Preimplantation Genetic Testing Specimen Kit contains two biopsy kits, each with 8 pre-labeled tubes and 1 negative control (labeled “NC”) tube.
- 3.4 Please **ensure the embryo biopsy barcodes on the worksheet correspond to the tube barcodes**. If you conduct the biopsy on two separate days, please use *one kit for each day*.
- 3.5 Label the top of each PCR tube with the appropriate embryo ID. Label the top of the “NC” tube with a “3”, “5”, or “6”, corresponding to the biopsy day. A “NC” tube is required for each biopsy day.

Note: If you are performing PGD, please make a control for each embryo biopsied. Please utilize the tubes in the biopsy kit and label them with the embryo number and the letter "C" for control (i.e., 1C, 2C, etc.). Please identify which PCR tubes were utilized as controls on the embryo biopsy sheet.

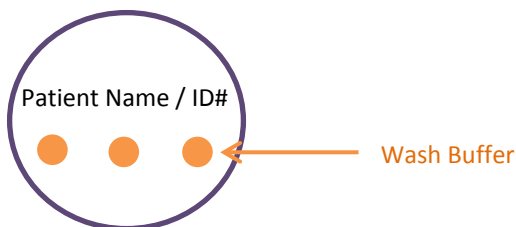
Materials Required

- CombiMatrix Preimplantation Genetic Testing kit (containing sterile embryo biopsy tubes, sterile negative control tube, sterile cell wash buffer, barcode labels)
- Stripper pipette with sterile tips
- Transfer pipette
- 35 mm sterile culture dishes
- Chilled 0.2 ml PCR tube rack
- Frozen ice pack (with flat surface)
- Dissecting microscope
- Sterile 10 µl filter tips
- Fine tipped permanent marker pen

- 3.6 In the hood, prepare sufficient number of culture dishes from the sterile packet, so there is one dish for each cell biopsy.
- 3.7 **Label the dishes** appropriately in accordance with your procedures on **both the lid and the base**.
- 3.8 In the hood, open the biopsy kit containing the sterile barcoded PCR tubes
- 3.9 Each biopsy requires one 0.2 ml sterile tube. Label the top of each tube with your internal embryo ID.
- 3.10 Once all the tubes are prepared, place them in a rack in the appropriate order.

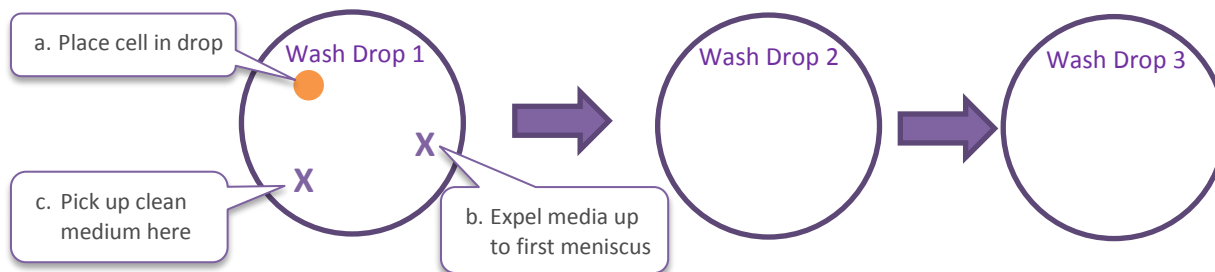
4. Cell Wash & Loading Protocol For Blastomere and Trophectoderm Cells

- 4.1 Place the dish containing biopsied cells on to the microscope stage. Ensure that the patient ID and the embryo number on the dish containing the cells matches the labeling on the PCR tubes.
- 4.2 Using a clean, sterile transfer pipette add three small drops (~15-20 µl) of cell wash buffer (provided in CombiPGS™ kit) in the culture dish and arrange them as shown below.



- 4.3 Attach a 135 µl pipette tip to a stripper. Dial down the stripper to “1” (this will assure that the PCR tube is not diluted with wash buffer, even if the whole stripper tip is pipetted into the PCR tube).
- a. If performing PGS, expel approximately 1 µl of wash buffer into a tube labeled “NC” and place in the biopsy kit.
 - b. If performing PGD, please prepare a control tube for each biopsy.**

- 4.4 Viewing under the microscope, pick up a cell from the dish in a minimal volume of media and place in the first droplet. Repeat a through c in cell Wash Drop 2 and 3.



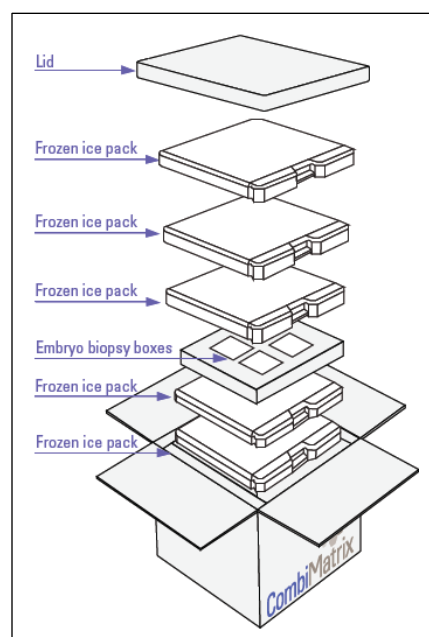
- 4.5 At Wash Drop 3 (step c), pick up the cell in a small volume of cell wash buffer. While holding the pipette, pick up the correctly labeled PCR tube, open the lid using the cap lip only and hold the tube sideways on the stage of the microscope.
- 4.6 Use the microscope to observe the pipette tip as it goes into the cell wash buffer within the tube; avoid touching the side of the tube with the pipette.
- 4.7 Gently expel the cell into the PCR tube, making sure to keep the final volume $<2.5 \mu\text{l}$. Remove the pipette from the tube, close the lid of the tube and place it in the chilled biopsy kit. Repeat steps for additional biopsy being sure to change pipette tips with each biopsy.
- Note:** The embryo biopsy can be loaded to an empty tube or loaded to a tube with a small volume of buffer; however, the final volume should not exceed $<2.5 \mu\text{l}$.
- 4.8 Complete the required fields on the embryo biopsy worksheet (found in the CombiPGS™ kit). Please note that the worksheet is pre-labeled with barcode IDs matching those of the tubes.
- 4.9 Once the biopsy is completed, place the tubes containing the biopsy specimens into the embryo biopsy kit, place into the plastic bag it originally came in, and place in the freezer for 20 minutes prior to shipping.

1. Return Shipping Instructions

Please do not ship on Fridays or Saturdays.

When the embryo biopsy tissue is ready for shipment, please assemble the items according to the steps outlined below:

- 1.1 Place two frozen ice packs inside the bottom of the styrofoam container. Ensure that the **ice packs** have been in the freezer for at least 24 hours and are **frozen solid at the time of shipping**. These are specialty ice packs designed to keep the specimen frozen up to 48 hours. Keeping the specimen frozen is essential to creating optimal conditions for analysis. If the ice packs are not frozen solid, they will not be able to maintain a frozen state for up to 48 hours, thereby diminishing the ideal condition.
- 1.2 Place the foam insert as the next layer.
- 1.3 Place the embryo biopsy box(es) in the clear bags within the pre-cut foam insert. A maximum of 4 embryo biopsy kits can be placed into each shipping box.
- 1.4 Add the three remaining frozen ice packs on top of the embryo container/protective foam.
- 1.5 Place the styrofoam lid on top of the styrofoam container and ensure the lid is pressed all the way down for a sealed fit.



- 1.6 Place all relevant paperwork such as the Test Requisition Form and worksheet, inside the clear pouch attached to the lid.
- 1.7 Fold the cardboard flaps and tape the box shut.
- 1.8 Ensure the FedEx label is not obstructed and the shipping address (CombiMatrix, 310 Goddard, Irvine, CA 92618) is clearly legible.
- 1.9 Call 800.GOFEDEx (800.463.3339) to schedule a pick-up or drop the box off at a FedEx location.
- 1.10 Please **email the FedEx tracking number to ivf@combimatrix.com**.

Note: Detailed shipping instructions can also be found on the shipping box and our website combimatrix.com. For additional guidance on how to ship the PGS specimen to CombiMatrix, please reference the video **CombiMatrix PGS Packaging Process** (https://youtu.be/P5xz_M3TWk8).

2. Ordering Additional PGS Sample Collection Kits and Shippers

- 2.1 Shipping kits may be ordered by contacting your CombiMatrix sales rep, by calling **Client Services** at **949.255.0920** or by emailing **ivf@combimatrix.com**.
- 2.2 Please include:
 - a. Number of kits requested (maximum order of 5)
 - b. Date needed by
 - c. Mailing address
 - d. Contact information

For more information, please contact:

CombiMatrix Client Services at **949.255.0920** or
email **ivf@combimatrix.com**