



**Standard Operating Procedure (SOP) for
24 Chromosome Aneuploidy Screening with aCGH
(Specimen Preparation and Shipping Protocol)**

Specimen Preparation and Shipping Protocol using Blastogen

This protocol prepares the followings:

- 1st polar bodies from unfertilized oocytes.
- 2nd polar bodies from fertilized oocytes.
- Single blastomeres from 3 day old embryos.
- Multiple Trophectoderm cells from 5/6 day old human embryos.

Review the instructions and if you have any questions do not hesitate to contact the Blastogen Lab Scientist at (310) 618-0618.

Blastogen will send a Specimen Collection Kit (SCK) when the Service team is notified via e mail lab@blastogen.com.

Please e mail (lab@blastogen.com) or call (310) 618 0618 us if you do not receive the SCK in 2 business day.

Supplies

Supplied by Blastogen:

- Array Washing Buffer (AWB) (Never open any AWB outside of a hood)
- Rack containing 20 sterile PCR collection tubes (Never open any of the tubes and Collection Tray outside of a hood)
- Styrofoam shipping box
- Dry ice Sticker

Supplied by Referring IVF clinic:

- Sterile petri dishes
- Stripper micropipette (Available at Origio MXL3-STR)
- Stripper Tip, inner diameter (e.g 75,100,125um) (Available at Origio #MXL 3-203-100um)
- 70% ethanol
- Dry ice to keep the samples frozen during shipping (depending on courier used)
- Permanent marker
- 2-20µL pipette and filtered pipette tips

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Note: For Eppendorf pipettors (Series 2000) we recommend 30ul filtered tips (Fisher Scientific, 02-707-33)

Upon Receipt, only WB needs to be placed in a Fridge (4 °C). Keep the rack containing 20 sterile PCR collection tubes at room temperature.

Specimen Preparation Best practices

- Never touch shipping tubes without gloves.
- Wear fresh gloves and face mask throughout the procedure.
- Change gloves regularly, especially if the gloves accidentally makes contact with a source of contamination, such as human skin.
- Use new Stripper tip for each specimen
- Blastogen recommends Intra cytoplasmic Sperm Injection (ICSI) for oocyte fertilization but is not required. With conventional IVF surplus sperm can remain and attached to the zona pellicuda and DNA from such may be accidently sampled at the time of biopsy which could potentially lead to misdiagnosis. Therefore washing step is very critical to avoid any sperm or cumulus cell contamination.
- Perform all steps in a laminar flow hood with air flowing.

Egg Retrieval and Embryo Biopsy

- E mail lab@blastogen.com for initial scheduling and changes to scheduling for egg retrieval date.
- During the embryo biopsy, if cell lyses occurs all tools should be changed before proceeding with next embryos.

Sample Preparation and Collection

- Once embryos are ready for biopsy, locate the Specimen Collection Kit (SCK).
- Sample preparation should be done in laminar flow hood and powder free gloves should be worn to prevent possible DNA contamination (Change gloves regularly, especially if the gloves accidentally makes contact with a source of contamination, such as human skin. Incubators, Scrubs, non-sterile equipment etc).

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- Prior to performing biopsy aliquot 5µl of Array Washing Buffer (AWB) into sterile PCR tubes for as many biopsy samples as you have.
Note: This step can be performed a day before the biopsy. Store the tubes in a fridge (4 °C).
- Never open any PCR tubes, Collection Tray or array washing buffer (AWB) outside of a hood.
- Use new pipette (stripper tip) for each sample to prevent possible contamination
- It is crucial to load the cells intact. Please make a note on embryo worksheet if there is full or partial lysis during the transfer.

Sample Washing and Loading Procedure

- Use a new, clean Stripper Tip for each sample
- Prior to performing biopsy, pipette four 15 µL drops (Array Washing Buffer) onto a sterile dish.
- Before removing the sample from the embryo biopsy dish rinse the Stripper Tip with 3 µl of fresh AWB by filling and then expelling 2 times.
- By using the same rinsed stripper tip transfer the sample to the first washing drop
- Flush 5µL of array washing buffer over the samples in order to move and roll it into a different area of the same drop. It is important to minimize the amount of fluid transferred from one wash drop to the next one.
- After the last washing step add biopsied cell to the sterile tube containing the array washing buffer and label the tube with the embryo number both on the cap and side of the tube
- Place the filled tubes into the provided rack. Place the lid onto the rack and seal the rack with a piece of laboratory tape.
- Label the box with the patients name, date of birth and the clinic name.

Shipping Specimens to BlastoGen

- Once all the samples have been collected in a tubes and placed in the rack, put the clear plastic top on the plastic tube tray. Tape all the side of the rack or around the rack to ensure it will not open during the shipment.
- Use insulated shipping box (Polyfoam container in a cardboard box) and place the rack with PCR collection tubes at the bottom of the container and fill out at least with 2.5 kg of dry ice and seal the shipping box.



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- If insulated shipping box and dry ice is not ready, ie when provided by courier, transfer the tube rack to a freezer (-15°C to -25°C) until box and dry ice arrive.
- Make sure to include the Biopsy worksheet in the shipping box.

All the packages should be sent to Blastogen to below address:

BlastoGen, Inc

Attention: Array Lab

406 Amapola Ave. Suite 225.

Torrance, CA, 90501

lab@blastogen.com

Phone: (310) 618-0618

Emergency Cell Phones: 818-919 1657 • 408-438 2899

Shipping Courier Options

Marken	1-800-MARKEN-1
Quick International	1-303-794 2757
Motorcycle Messenger	1-800-282-1ECS(327)
Fedex	1 800-GOFEDEX