# BLASTOCYST TROPHECTODERM BIOPSY

Directions for supplementation of un-supplemented G-Series™ media can be found in the G-Series Manual on www.vitrolife.com. Once supplemented, the media should be used as the G-Series PLUS media described below.

G-2™ PLUS or G-TL™

OVOIL™

G-MOPS™ PLUS

**Note:** It is recommended to biopsy only advanced blastocysts with an obvious ICM and trohectoderm.

## Preparations

**1.** On Day 3, using a laser, make a small hole or channel (~5 µm wide) in the zona pellucida of the embryo. Commonly, on Day 5/6 a few trophectoderm cells will herniate out of this hole. This step is optional but will facilitate the biopsy procedure.

**Note:** If cells are to be biopsied from cryopreserved blastocysts, perform this step immediately after thawing and before the blastocyst has re-expanded.

**2. Culture dish:** Place droplets of G-2 PLUS or G-TL in a culture dish tested and approved for IVF, add 2 droplets for washing and cover with OVOIL™.

**3.** Label the droplets with consecutive numbers for all blastocysts to be biopsied. Place the dish in the $CO_2$-incubator for

**6 h or overnight**

**4. Biopsy dish:** Place an appropriate number of 4-6 µL droplets of G-MOPS PLUS in a dish tested and approved for IVF. Make one droplet per blastocyst to be biopsied. Cover the droplets with OVOIL.

**5.** Label the droplets with consecutive numbers for all blastocysts to be biopsied, the same as for the culture dish. Place the dish on a heated stage or in an incubator without $CO_2$ until

**+37°C has been reached**

**Note:** To maintain correct pH, G-MOPS PLUS should only be warmed at +37°C. G-MOPS PLUS must **never** be equilibrated with $CO_2$.

## Biopsy procedure

On day 5 or 6 a few cells should be herniating out of the hole in the zona (thawed blastocyst will have cells herniating out of the zona after 2 to 3 hours of re-expansion).

**1.** Under the inverted microscope, use a holding pipette to support the blastocyst. Aspirate 3-6 of the herniated cells into the biopsy pipette. The biopsy pipette should have an inner diameter of ~20-30 µm.

**2.** Using a laser fire the laser at the constricted area of cells at the end of the biopsy pipette. Two or three times are usually enough. Gently pull to separate the group of cells from the rest of the blastocyst.

**3.** Deposit the biopsied cells at the bottom of the biopsy droplet and move the blastocyst to the top of the droplet. Biopsy time should not exceed 2-3 minutes.

**4.** Repeat until all blastocysts have been biopsied.

**5.** Rinse the blastocyst in the wash droplets in the culture dish and place it into the numbered culture droplet of the culture dish.

**Note:** From this point on, all blastocysts must be cultured individually in numbered droplets.

**6.** Leave the biopsied cells in the droplets of the biopsy dish until all blastocysts have been biopsied.

**7.** After all blastocysts have been biopsied, continue with genetic analysis of cell samples according to the diagnostic tool manufacturer´s instructions.

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