

EMBRYO CULTURE USING G-TL

and Primo Vision time-lapse embryo monitoring system

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.

Day 0



G-TL™



OVOIL™

Prepare the Primo Vision culture dish by priming each well with G-TL medium. Load each well separately and then add 80 microlitres of G-TL to cover all the wells. Note that the medium drop should be as flat as possible and that air bubbles must be avoided. Add two wash droplets of G-TL and cover with OVOIL and equilibrate at

**37°C 6 % CO₂
overnight**

The rim around the matrix of micro wells does not mark the edge of the proposed droplet of culture medium. The role of the rim is to stabilize the movement of the medium so that the embryos stay in the wells.

When preparing a droplet, spread it out over the edge of the rim and try to achieve a flat dome.



G-MOPS™ PLUS

Warm G-MOPS PLUS (for fertilisation assessment) in rinsed tightly capped tubes in a warming incubator **without CO₂*** at

37°C overnight

* An adequately calibrated warming block can be used for tubes instead of a warming incubator.

Ensure that the denudation and washing procedures are performed at 37°C

Day 1

1. Fertilisation assessment

For standard IVF, if denudation and fertilisation assessment can be performed within 2 minutes, this can be done in the insemination dish. Otherwise, transfer the oocytes to a centre well dish with pre-warmed G-MOPS PLUS before starting the denudation procedure.

Remove cumulus and corona cells from the oocytes using a denudation pipette and assess fertilisation at

37°C



For ICSI oocytes, assess fertilization in the G-1 PLUS culture dish or in the G-TL dish.

2. Culture

Check the Primo Vision culture dish for air bubbles and remove them if present.



Wash the zygotes in G-TL wash droplets in the Primo Vision culture dish prepared on Day 0 and transfer the zygotes to the wells, one zygote per well. Culture at

37°C 6 % CO₂

Day 2 - 5

Assessment

Assess embryo development using Primo Vision time-lapse system.

For embryo transfer, see separate Embryo transfer protocol.