# Embryo culture G-1 PLUS and G-2 PLUS

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-1™ PLUS


OVOIL™

Prepare micro-droplet culture dishes with 25 µL droplets of G-1 PLUS for washing and for culture. Cover with OVOIL and pre-equilibrate at

**37°C 6 % $CO_2$ overnight**


G-MOPS™ PLUS

Warm G-MOPS PLUS (for fertilisation assessment) in rinsed tightly capped tubes in a warming incubator **without $CO_2$* at**

**37°C overnight**

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

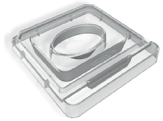
**Ensure that the denudation and washing procedures are performed at 37°C**

## Day 1

### 1. Fertilisation assessment

For inseminated oocytes, transfer the oocytes to a centre well dish with pre-warmed G-MOPS PLUS. If denudation and fertilisation assessment can be performed within 2 minutes, G-IVF™ PLUS can be used instead of G-MOPS PLUS.
Remove cumulus and corona cells from oocytes using a denudation pipette and assess fertilisation at

**37°C**



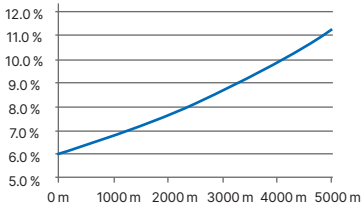
For ICSI oocytes, assess fertilisation in the G-1 PLUS micro-droplet culture dish.

### 2. Culture


Wash the zygotes extensively in the G-1 PLUS micro-droplet dishes prepared on Day 0 and transfer the zygotes to 25 µL G-1 PLUS culture droplets covered with OVOIL. Culture at

**37°C 6 % $CO_2$ overnight or for 2 days**

If your clinic is located at a higher altitude than sea level, $CO_2$ percentage should be increased, see graph below.




## Day 2

### Assessment

Assess embryo cleavage.

**For embryo transfer day 2, see separate Embryo transfer protocol**

## Day 3

### Assessment

Assess embryo cleavage.

**For embryo transfer day 3, see separate Embryo transfer protocol**

**We recommend G-MOPS PLUS for washing and assessment of embryos before transfer to G-2 PLUS or EmbryoGlue®**

## Blastocyst culture

### 1. Prepare micro-well dishes for Blastocyst culture


G-2™ PLUS


OVOIL™

In the morning of day 3, prepare micro droplet culture dishes with 25 µL droplets of G-2 PLUS for washing and for culture. Cover with OVOIL and pre-equilibrate at

**37°C 6 % $CO_2$ ≥ 6 h**

### 2. Move embryos to G-2™ PLUS


In the afternoon of day 3, wash the embryos extensively in equilibrated G-2 PLUS droplets and transfer the embryos to G-2 PLUS culture droplets, maximum 5 embryos per droplet. Culture at

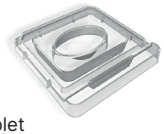
**37°C 6 % $CO_2$ 2 days**

## Day 4

### Prepare micro-droplet culture dishes


G-2 PLUS

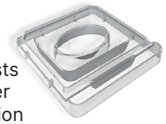

OVOIL


Prepare centre well dishes with fresh G-2 PLUS.
Prepare micro-droplet dishes for prolonged culture if needed and pre-equilibrate at

**37°C 6 % $CO_2$ overnight**

## Day 5

### In the morning of day 5


Assess embryo cleavage, and move the blastocysts selected for transfer and cryo preservation to the equilibrated G-2 PLUS centre well dishes and leave at

**37°C 6 % $CO_2$ until 10-30 min before transfer**

**For blastocyst transfer, see separate Embryo transfer protocol.**



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