

ICSI PROCEDURE

Day 0

1. Prepare culture dishes



G-TL™ or G-1™ PLUS



OVOIL™

Prepare dishes with G-TL or G-1 PLUS and equilibrate at

37°C 6 % CO₂ 6 h



G-RINSE™

When applicable, pre-rinse all utensils, including dishes and pipettes with G-RINSE.

**Never place any G-MOPS™ product in a CO₂ incubator
Do not use OVOIL equilibrated in a CO₂ environment when covering any G-MOPS product**

2. Prepare denudation dishes



HYASE™-10x



G-MOPS PLUS



OVOIL

Dilute HYASE-10x with G-MOPS PLUS

Prepare rinsed 5-well

denudation dishes with HYASE-10x

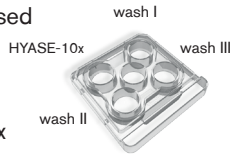
diluted in

G-MOPS PLUS and with G-MOPS PLUS for wash, cover

with OVOIL and warm at

37°C 15 min

Use within one hour.



3. Prepare ICSI dishes



ICSI™



G-MOPS PLUS



OVOIL

Prepare ICSI dishes with ICSI and

G-MOPS PLUS droplets covered

with OVOIL and warm in a warming incubator

without CO₂ at

37°C 15 min

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



4. Denudation

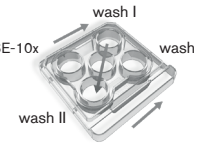
Denude the oocytes in

HYASE-10x

and wash

three times in

warmed G-MOPS PLUS



Exposure to hyaluronidase for longer than 30 seconds may damage the oocytes

5. ICSI

Add sperm to the ICSI droplet.

Transfer the denuded oocytes

to G-MOPS PLUS droplets. Place one oocyte per droplet

and maximum four oocytes at a time, and inject the oocytes.



Adjust temperature of the heated stage used for ICSI to maintain 37°C in the droplets

6. Wash and culture

Wash the injected oocytes in

several droplets

of G-TL or

G-1 PLUS and transfer

to fresh G-TL or G-1 PLUS droplets for culture at

37°C 6 % CO₂ overnight

For fertilisation assessment and culture, see separate Embryo culture protocol.

