

ICSI PROCEDURE

Day 0

1. Prepare culture dishes



Gx-TL™



OVOIL™

Prepare dishes with Gx-TL 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 Gx-MOPS PLUS product in a CO₂ incubator. Do not use OVOIL equilibrated in a CO₂ environment when covering Gx-MOPS PLUS

2. Prepare denudation dishes



HYASE™-10X



Gx-MOPS PLUS



OVOIL

Dilute HYASE-10X with Gx-MOPS PLUS

Prepare rinsed 5-well

denudation dishes with HYASE-10X

diluted in Gx-MOPS PLUS and with Gx-MOPS PLUS for wash, cover with OVOIL and warm at

37°C 15 min

Use within one hour.

3. Prepare ICSI dishes



ICSI™



Gx-MOPS PLUS



OVOIL

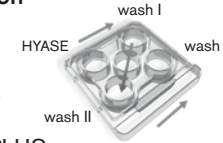
Prepare ICSI dishes with ICSI and Gx-MOPS PLUS droplets covered with OVOIL and warm in a warming incubator **without CO₂** at

37°C 15 min

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

4. Denudation

Denude the oocytes in HYASE-10X diluted in Gx-MOPS PLUS and wash three times in warmed Gx-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 Gx-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 Gx-TL and transfer to fresh Gx-TL droplets for culture at

37°C 6 % CO₂ overnight

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