



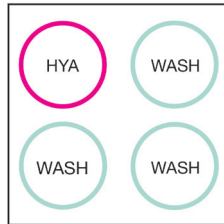
GM501 Hyaluronidase



Preparation for denudation of fresh oocytes

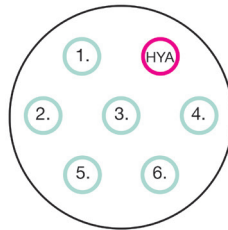
Preparation of the 4-well dish

- One 4-well dish ("Hya-dish") needs to be prepared for 10 oocytes.
- First fill well 2 to 4 with 400 µl GM501 Wash medium and cover the filled wells with GM501 Mineral Oil. Equilibrate the dish overnight in a humidified CO₂ incubator.
- Warm the GM501 Hyaluronidase and fill the first well with 400 µl warmed GM501 Hyaluronidase.



Preparation of microdrop dish

- One 60 mm Petri dish ("Hya-dish") needs to be prepared for 10 oocytes.
- Each dish contains a total of 7 drops, 80 µl each (6x GM501 Wash medium, 1x GM501 Hyaluronidase). In the diagram the wash drops are shown in green and the hyaluronidase drop is shown in pink.
- To facilitate identification mark the spot for the Hyaluronidase drop on the bottom of the Petri dish. First add the 6 drops with GM501 Wash medium to the dish and cover with GM501 Mineral Oil. Equilibrate the dish overnight in a humidified CO₂ incubator.
- Warm the GM501 Hyaluronidase and add one drop of 80 GM501 Hyaluronidase to the dish at the marked position.



Denudation procedure using the microdrop dish

1. Pipette up to 10 oocytes into the first drop ("1") of the dish.
2. Transfer 5 oocytes to the "Hya" drop.
3. Pipette the oocytes immediately up and down (5 to 10 times) using for example a pipette with 100 µl tip (MEA-tested) adjusted to 50 µl. The cumulus cells will detach and the oocytes still surrounded by corona cells will be visible.
ATTENTION: The oocytes should not be in the Hyaluronidase for more than 30 seconds!
4. Pick up the oocytes using the denudation pipette (inner diameter 125-155 µm) and transfer them to the next GM501 Wash containing drop ("2"). Aspirate and blow back the oocytes repeatedly to remove residual hyaluronidase.
5. Transfer the oocytes to the next GM501 Wash containing drop ("3"). Aspirate and blow back the oocytes repeatedly until nearly all corona cells are removed.
6. Transfer the oocytes to the next GM501 Wash containing drop ("4"). Leave the denuded oocytes in this drop.
7. Repeat steps 2 to 6 with the remaining oocytes.
8. When all oocytes are collected in GM501 Wash drop "4" and are free of cumulus and corona cells, wash them in the last two drops ("5" and "6").
9. The denuded oocytes can then be transferred to a dish containing GM501 Cult for further incubation until ICSI is performed or directly to an ICSI dish.

Gi128/V3

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