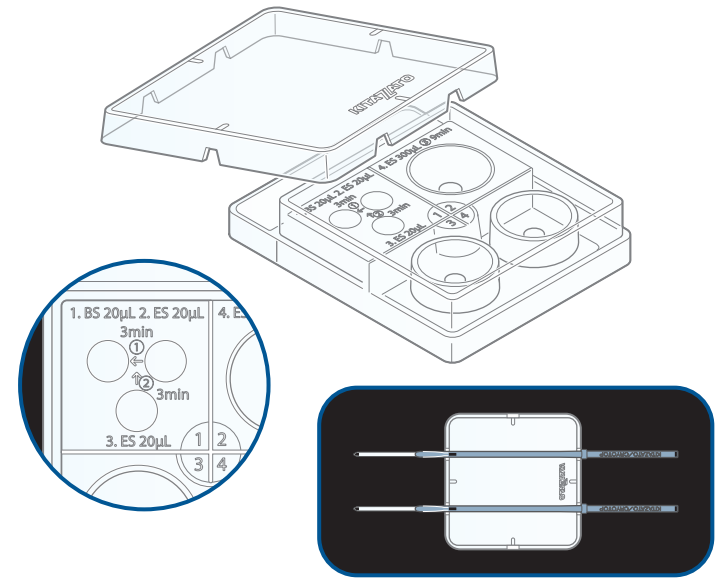
PRODUCT CATALOGUE

Oocyte Cryo Plate



- Premarkings on the plate guide you to prepare the different solutions in appropriate volumes.
- High repeatability in the equilibration conditions leads to stable results.
- Ditches on the lid hold Cryotop stable for better handling.

Collaborative Development : Kato Ladies Clinic / Keio University, Department of. Obstetrics & Gynecology / National Center for Child Health and Development / Obstetrics and Gynecology, St Marianna University School of Medicine / Obstetrics and Gynecology, Graduate Schoold of Medicine and Faculty of Medicine, The Univertisy of Tokyo

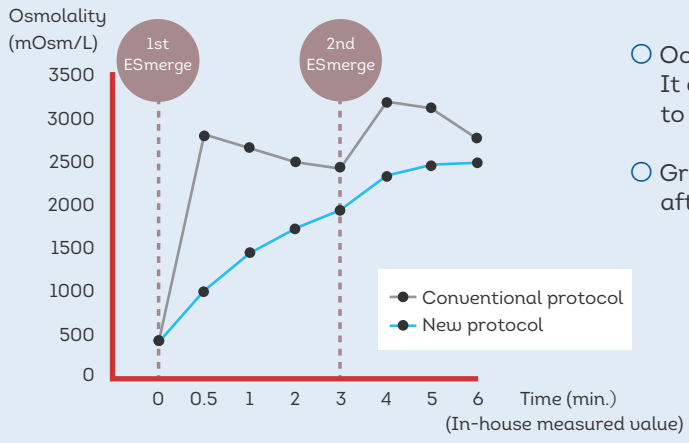
REF	Code	Contents
83061	Oocyte Cryo Plate	10pcs/pk

QUALITY CONTROL

Sterility Test / Endotoxin ≤0.5EU/unit(EU/mL) / Mouse Embryo Assay ≥80%

RESEARCH DATA

Comparison of osmolality change between the conventional and the new protocol with the Oocyte Cryo Plate.



(In-house measured value)

- Oocyte Cryo Plate allows solutions to mix moderately. It allows oocytes to equilibrate more gradually compared to the conventional protocol.
- Gradual osmolality change improve oocyte development after warming.

Specification may change without pre-notice for purpose of product improvement.

Kitazato Corporation

HEADQUATER : 100-10 Yanagishima, Fuji, Shizuoka 416-0932 JAPAN

TOKYO : 1-1-8 Shibadaimon, Minato-ku, Tokyo 105-0012 JAPAN

Mail contact@kitazato.co.jp

PROTOCOL

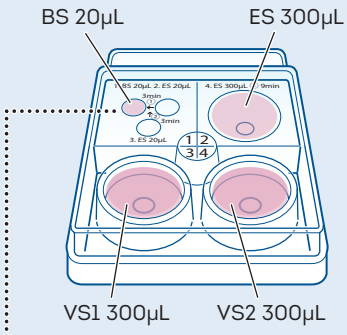


Fig.1

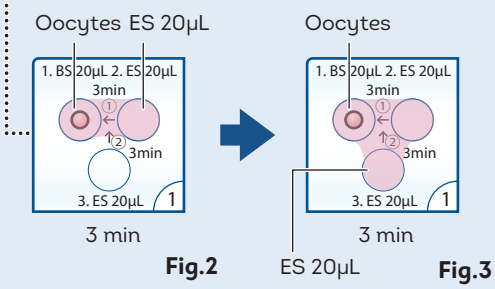


Fig.2 Fig.3

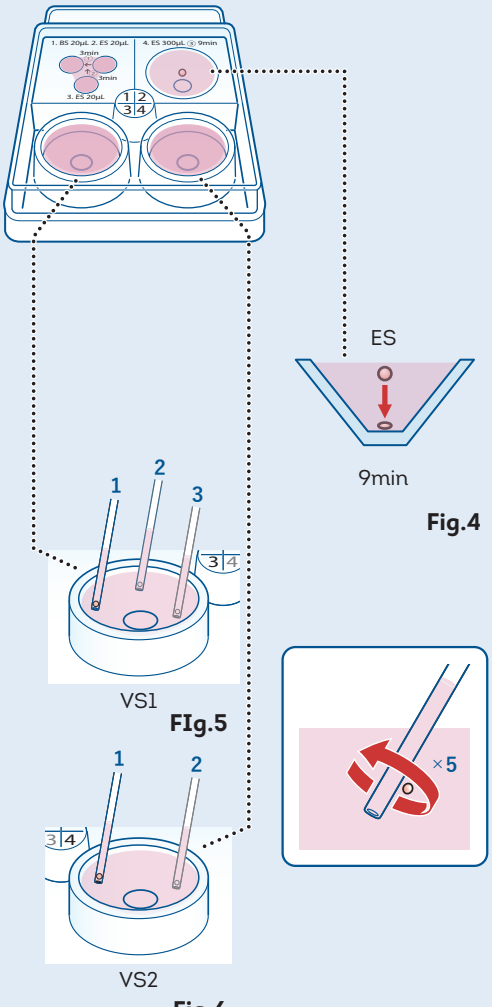


Fig.4 FIg.5 Fig.6

The video protocol is available on our official YouTube channel.

Perform the following pre-freezing procedures using our oocyte/embryo vitrification solution.

01 On Oocytes Cryo Plate, dispense 20 μL drop of BS in area 1, 300 μL of ES in area 2, 300 μL of VS in area 3 and 4 respectively. Keep the lid on until use (Fig.1).

02 Transfer the oocytes from the culture medium to the BS drop.

03 Dispense 20 μL of ES drop on the right side of the BS drop, and merge the ES to the BS drop using the chip of the pipette, then leave it still for 3 min (Fig. 2).

04 Dispense 20 μL drop of ES below the mixed drop. Merge the new ES drop to it with the chip, and leave it still for 3 min (Fig. 3).

05 Transfer the oocytes to the surface of the dispensed ES in area 2 and leave it still for 9 min (Fig. 4).

06 After completion of ES equilibration, aspirate the oocytes/embryos with minimal volume of ES at the tip of the pipette .
Transfer the aspirated oocytes /embryos to the surface of VS1 and set the timer.

*Referential operation time in VS1 and VS2 together is 60 - 90 seconds to ensure dehydration.
It is recommended to keep oocytes/embryos immersed in VS for at least 60 - 90 seconds.

07 Expel the remaining ES in the pipette to the outside the well, and wash the pipette by aspirating and expelling sufficient volume of VS.

08 Aspirate the oocytes/embryos and perform steps the below ① and ② in three positions in the VS1.
① Expel the oocytes/embryos from the pipette to the VS.
② Stir around the oocytes/embryos 5 times gently for 5 seconds.
The oocytes/embryos are equilibrated and dehydrated by performing steps ① and ② in three different positions (Fig. 5).

09 Wash the pipette with VS2 as explained in STEP2.
Perform ① and ② of step **08** twice at different positions in the VS2.
Confirm the completion of VS equilibration by the below two criteria before loading oocytes/embryos on Cryotop.

- The oocytes/embryos are shrunk.
- The oocytes/embryos does not rise to the surface. (They stay in focus under microscope.)

If the above two points are not confirmed, perform ① and ② again in no rush (Fig. 6)..