KITAZATO®

VITRIFICATION KIT

-Cryotop Method -

Vitrification Protocol for Oocyte

Kitazato BioPharma Co.,Ltd.

Editor by Masashige Kuwayama Ph.D
Advanced Medical Reseach Institute of Fertility
KATO LADIES CLINIC

Reference)
Kuwayama, M. Highly efficient vitrification method for cryopreservation of human oocytes. RBM Online. Vol.11 (3), 300-308 2005.

1. Preparation -Vitrification-

- Vitrification Kit

  Step 1 (ES; Equilibration Solution)

  Step 2 (VS; Vitrification Solution)

  WS; Washing Solution

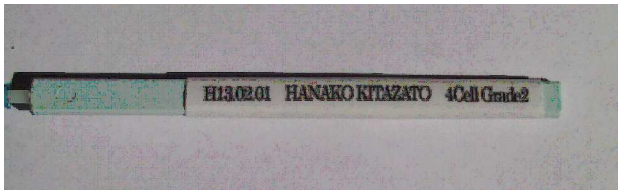
- Microscope
- Petri dishes(35mm) **ex.FALCON1008, 351006**
- Liquid N_2
- L N_2 Container **Rack Cooling(Volume of 1 ~ 2L)**
- Stopwatch or Timer
- Tweezers
- Pipette Glass (**diameter of an oocyte at size; about 150μ m)**

2. Vitrification Protocol

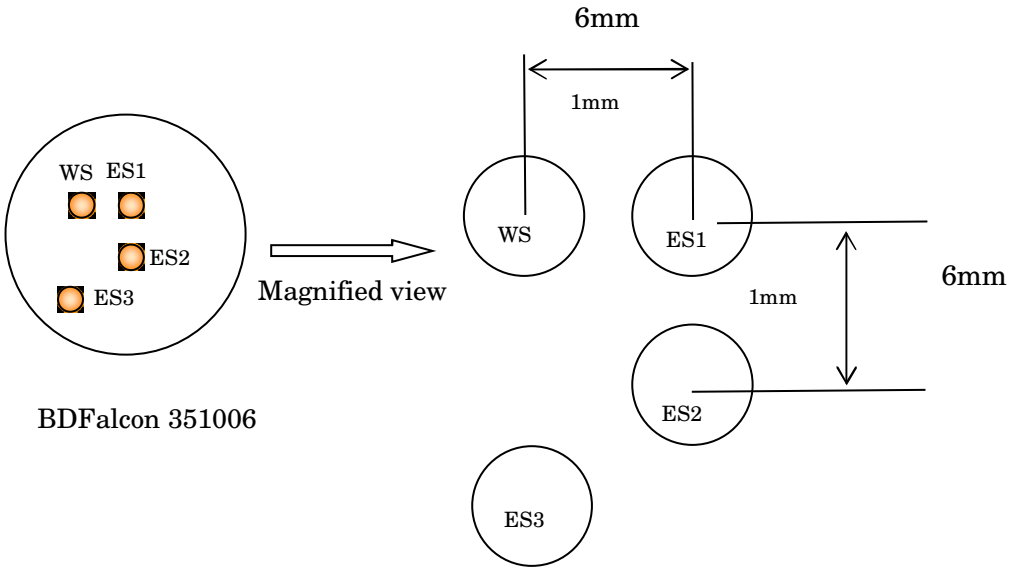
1. Warm ES and VS to room temperature (25-27℃).

2. Write WS , ES or VS on the bottom surface of 35mm-dishes respectively. Then turn tubes (except for TS) upside down two times to mix the solutions, and pour full content into each dish.

3. Write necessary information on the bar of Cryotop.



4. Make three droplets, one 20¼ WS droplet and two 20¼ ES droplets, on a 50mm-dish at 1mm intervals. One millimeter interval can be created by making 6mm intervals between center points. And make a droplet of ES3 at lower left of ES2 droplet. Then cover the dish immediately after making droplets.

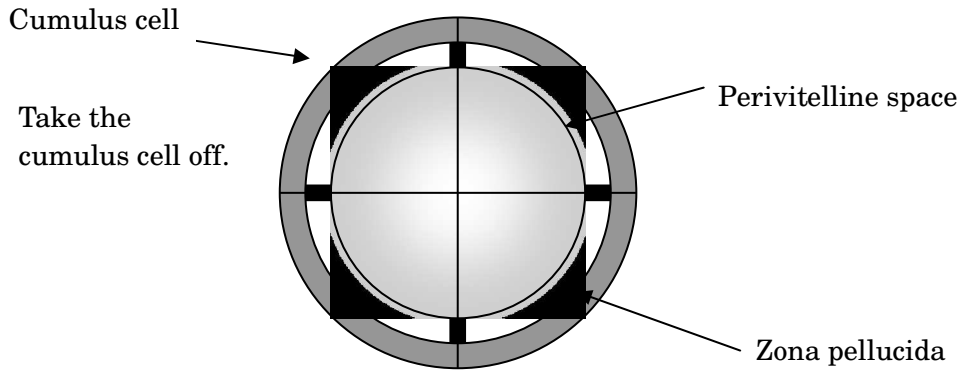


5. Take the culture dish containing an oocyte from the incubator.

6. Check the oocyte under a microscope morphologically and record the quality and any information.

*Low quality oocytes are not appropriate for cryopreservation (vitrification).

Observe the embryo rolling it with a pipette.



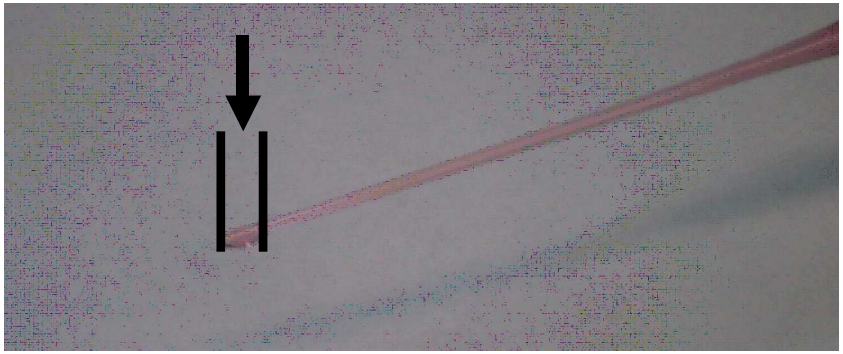
Important

▬: Compare the width of perivitelline space at these four points with thickness of zona pellucida and keep it in mind (ex. 1:1). It allows seeing the state of dehydration and equilibrium after immersing the embryo in ES.

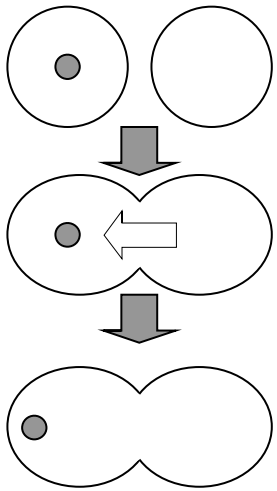
7. Fill the container with fresh liquid nitrogen (~ 90% full).

8. Aspirate the oocyte within the end of a capillary..

9. Transfer the oocyte onto the surface of WS with minimum medium.



10. Combine the ES1 droplet and the WS droplet using a new pipette (move the pipette from the left edge of ES1 droplet to the right edge of WS droplet) and leave at rest for 3 minutes.



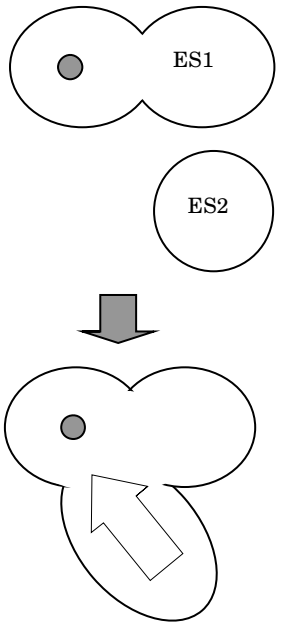
Move the pipette from the center of ES1 to that of WS.

ES1 droplet flows to the direction of WS.

The oocyte moves to the left side of WS droplet.

The following steps are operated timing with a stop-watch.

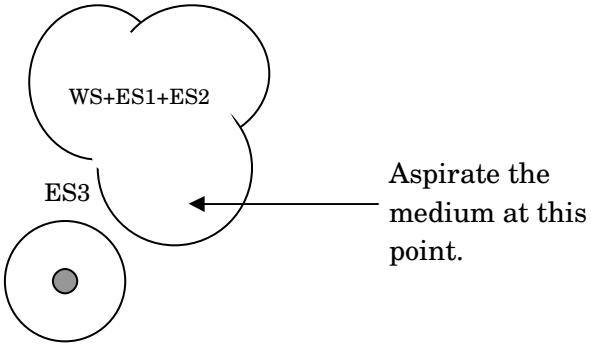
11. Three minutes later, combine the WS+ES1 droplet and ES2 droplet and leave it for 3 minutes at rest.



Move the pipette from the center of ES2 to that of WS.

The droplet flows to the direction of WS.

12. Aspirate ES2 medium with a new pipette at first, then aspirate the oocyte and medium enough for 3 oocytes. And expel it onto the surface of ES3 droplet.



For equilibration, the oocyte volume is required to be recovered completely.

The exposure time in ES3 is up to a maximum of 9 minutes. The time required for recovery depends on the vitality of the oocyte.

The following steps from 13 to 18 should be completed within 1 minute.

13. Aspirate the oocyte with minimum volume of ES solution using a capillary and keep it within the end.

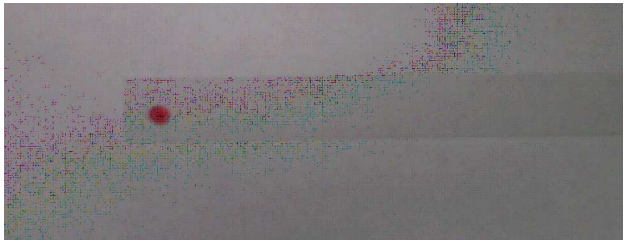
14. Put the oocyte on the surface of VS and expel any remaining ES solution out of the capillary.

15. Aspirate the embryo with the capillary and pour out into fresh VS. Repeat this operation three times at different position in VS solution.

16. Aspirate the oocyte within the end of the capillary and put it beside the black mark on the Cryotop sheet with minimum amount of VS solution (0.1¼ or less)

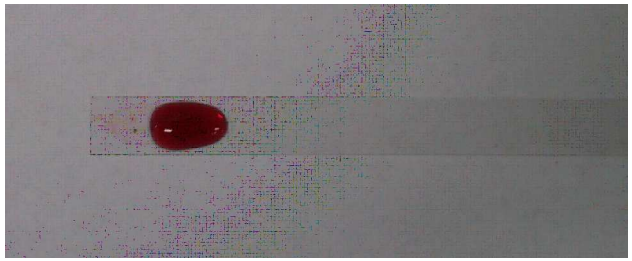
Good example

Droplet is made planar on the distal end of Cryotop.

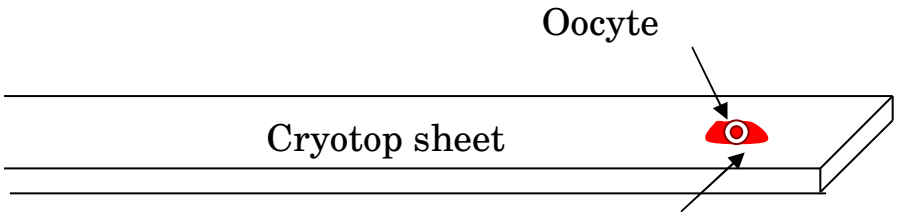


Bad example

Droplet is made steric.



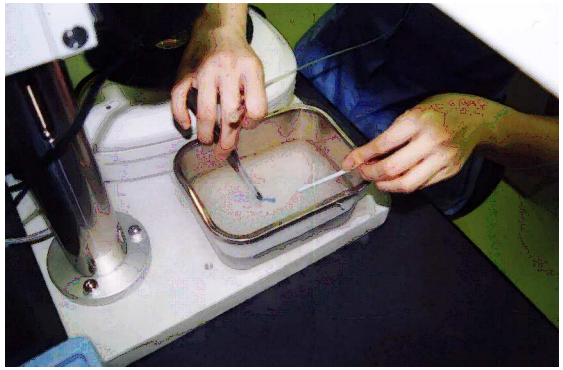
17. Check if the embryo is on the sheet under the microscope.

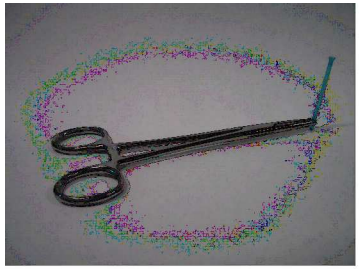


Minimum volume of VS

18. Plunge the Cryotop sheet into liquid nitrogen immediately. Keep it in liquid nitrogen until transferring to a storage tank.

19. Hold the straw cap with tweezers in liquid nitrogen and put the Cryotop in it tightly.





Straw holder
Code: VT-SH15

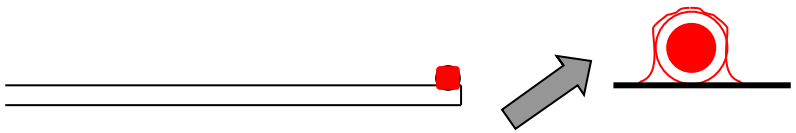
20. Check if the cap is fixed to Cryotop and put it into the cane. Then transfer it to the storage tank for long time storage.

【Instructions】

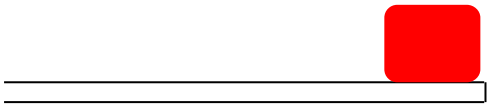
1. It is recommended that **one Cryotop is used for one oocyte.**

2. Refer to the examples below for droplet formation of vitrification solution (VS).

- **Good example** ・・・ Planar droplet



- **Bad example** ・・・ Steric droplet



3. Preparation -Thawing-

- Vitrification Kit

  Step 1 (TS; Thawing Solution)

  Step 2 (DS; Diluent Solution)

  Step 3 (WS1; Washing Solution 1)

  Step 4 (WS2; Washing Solution 2)

- Microscope
- Four petri dishes(35mm) ex. FALCON 1008
- Liquid N_2
- Rack cooling
- Stopwatch or timer
- Pipette glass
- Tweezers

4. Thawing Protocol

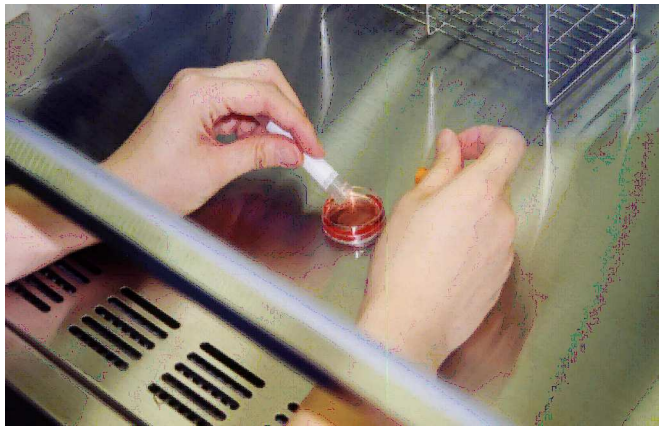
1. Warm TS tubes with caps and dishes to 37℃ in an incubator.

   *Write TS on the bottom of the dishes before warming.

2. Transfer the cane into a container filled with fresh liquid nitrogen. Then take out the Cryotop from the cane handling in the container.

   *Put the container beside a microscope.

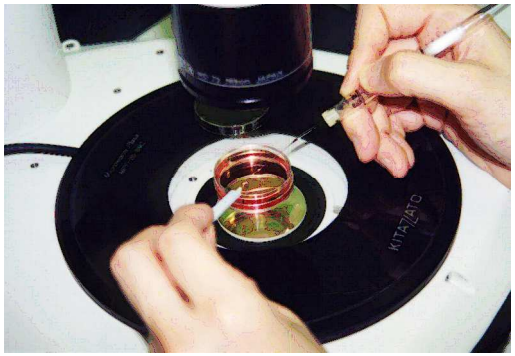
3. Write DS , WS1 or WS2 on the bottom surface of each 35mm-dish. Turn tubes (except for TS) upside down two times to mix the solutions, and pour full content into each dish.



The following steps are operated timing with a stop-watch

4. Retrieve the straw cap from Cryotop with tweezers handling in liquid nitrogen..

5. Quickly plunge the sheet of Cryotop into TS.



6. After the oocyte comes off of the sheet, aspirate it within the end of capillary and put on the center of the dish.

7. Aspirate the oocyte with the capillary at 1 minute after putting into TS.

8. Transfer the oocyte to the bottom of DS with small amount of TS.

9. The oocyte will swell temporally and begin to shrink in DS in 3 minutes.

10. Transfer the oocyte onto the bottom of WS1 dish with small amount of DS and keep for **5 minutes in WS1**.

11. Transfer the oocyte onto the surface of WS2 dish with minimum amount of WS1, then keep for **5 minutes in WS2** on a plate warmer (37℃).

12. Transfer the oocyte into the culture medium.

-END-

KITAZATO®
Kitazato BioPharma Co., Ltd.
278-7 Mitojima, Fuji-city, Shizuoka, Japan Zip 416-0921
Tel.+81-545-66-2202 Fax.+81-545-60-5772
E-mail. info@kitazato-biopharma.com