THE CRYOTEC METHOD

Manual Book

ÑFor Oocyte and Embryoò

Vitrification

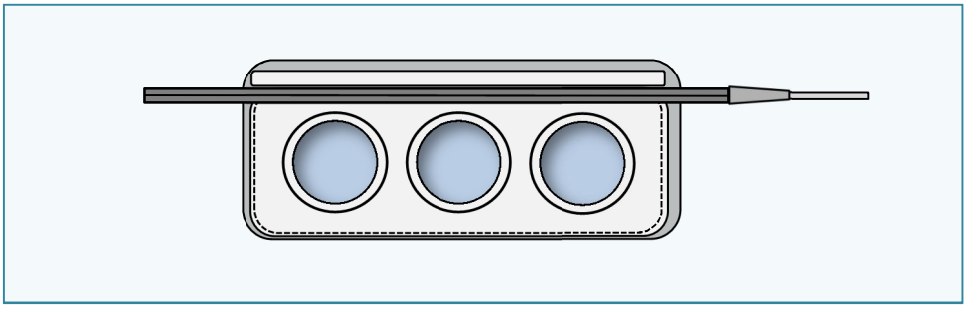
Materials

- **Cryotech Vitrification Kit**
  - Equilibration Solution (ES): 1 vial of 1.0 ml
  - Vitrification Solution (VS): 2 vials of 1.0ml
  - 4 Cryotecs
  - 3 Vitri Plates with 3 wells each
- Microscope (Turn off the heating plate)
- Stop watch (with count up function)
- Tweezers / Scissors
- Micro pipette for 300㎕

Preparation

1. Bring ES and VS vials to room temperature (25~27°C) at least 1 hour before vitrification.
2. Open a Cryotec package with a scissor. Write information of oocyte/embryo on the handle of Cryotec, and set on Vitri Plate (Fig.1).

Fig.1. Vitri Plate with Cryotec



3. Prepare fresh liquid nitrogen.
4. Take the culture dish containing oocyte/embryo out from the incubator.

Equilibration (12 ï 15 min)

1. Fill the wells of Vitri Plate with 300㎕ ES and 300㎕ VS, respectively (Fig. 2).
   Put the lid on the Vitri Plate immediately.

Fig. 2. Preparation of each solution

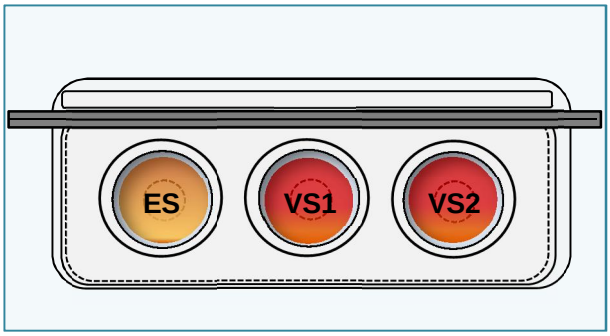
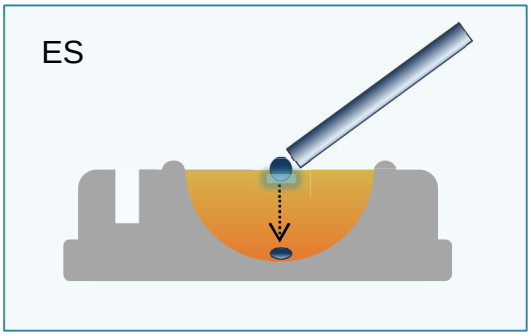


Fig. 3. ES Equilibration

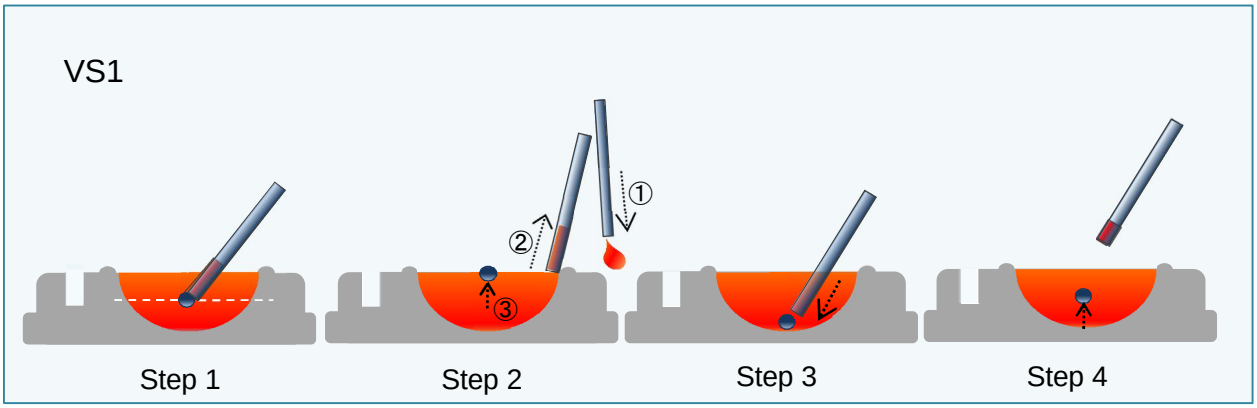


2. Aspirate the oocyte/embryo at the middle of the fine part of a pasteur pipette.
3. Put the oocyte/embryo with small amount of medium on the surface of ES well.
   Start the stop watch. As oocyte/embryo sink to the bottom, it would shrink (Fig. 3).
4. Put the lid and wait for the recovery of the shrinkage. When the oocyte/embryo volume is completely recovered, it is the end of this step.

   If you cannot confirm the complete recovery, the limit time of this step is 15 min to oocyte and blastocyst (160-200 μm in diameter), and 12 min to 4 -8 cells embryo.

Vitrification 1 (30 - 40 sec)

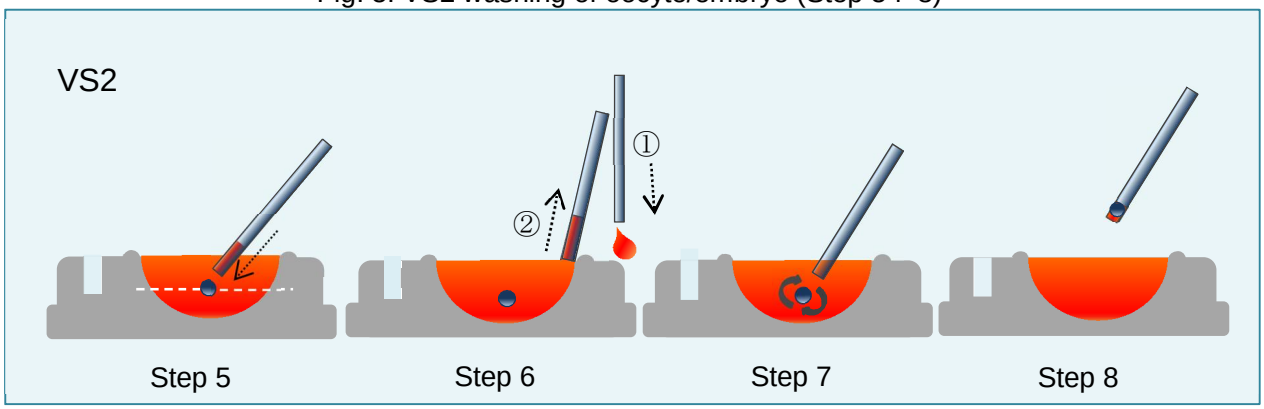
Fig. 4. VS 1 washing of oocyte/embryo (Step 1 ~ 4)



1. Aspirate the oocyte/embryo and **ES** until the middle of the pipette.
2. Blow out oocyte/embryo at the middle depth of **VS1** with **ES** (Step 1).
3. Expel remained **ES** inside the pipette (Step 2/①) and aspirate fresh **VS1** from the edge of the wall (Step 2/②**)**.
4. Oocyte/embryo floats immediately to the surface of **VS1** (Step 2/③).
   Aspirate oocyte/embryo at the top inside of pipette.
5. Blow it again to the bottom of **VS1** (Step 3).
6. The oocyte/embryo floats slowly to the middle depth and stop (Step 4).
7. Expel remained **VS1** inside the pipette, and aspirate fresh **VS2**.
   Aspirate oocyte/embryo at the top inside of the pipette.

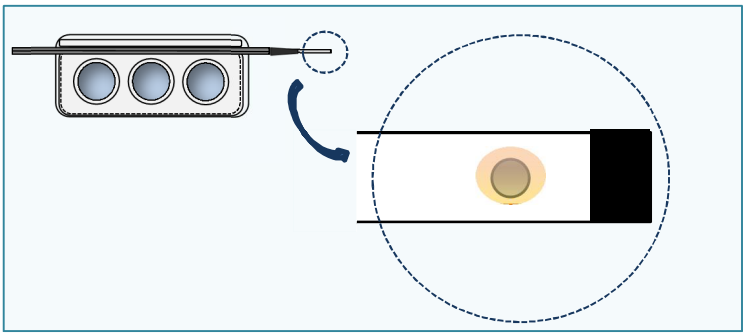
Vitrification 2 (10 ï 20 sec)

Fig. 5. VS2 washing of oocyte/embryo (Step 5 ï 8)



8. Blow the oocyte/embryo to the middle depth of **VS2** (Step 5).
9. Expel remained **VS** inside the pipette (Step 6/①) and aspirate fresh **VS2** from the edge of the wall (Step 6/②).
10. Blow **VS2** and mix the solution around oocyte/embryo to exchange the remaining previous solution (Step 7).
11. Expel and wash inside of the pipette with fresh **VS2**.
12. Take oocyte/embryo at the top inside of pipette (Step 8).
13. Place the oocyte/embryo near the end of Cryotec sheet with minimal volume of **VS2**. (1 oocyte/embryo per droplet is recommended) (Fig. 6).
14. Immediately submerge the Cryotec into fresh liquid nitrogen.
15. Put the straw cap on Cryotec in the liquid nitrogen.

Fig. 6. Oocyte/embryo on the Cryotec



Note 1

Use a right size pasteur pipette.

- 140-150 ɛm for oocyte and cleavage stage embryo.
- 160~200 ɛm for blastocyst.

Note 2

Best timing of vitrification of blastocyst: the size(diameter) should be between160~220 ɛm for perfect survival after vitirfication.

Note 3

All the solutions only allowed to be used within 30 days after uncorking, and on the condition that dropping method is aseptic.