Vitrification Protocol for Oocytes / Embryos

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Vitrification Procedure

PART 1 Materials Required

1. Vitrification Media VT601 / VT621 [Ref. 91101 / 91207]
   No.0 BS (Basic Solution) 1.5mL vial ×1*1
   No.1 ES (Equilibration Solution) 1.5mL vial ×1
   No.2 VS (Vitrification Solution) 1.5mL vial ×2
   ※Equilibrate all the solutions to room temperature before use
2. Shrinkage Solution VT505-4 / VT525-4 [Ref. 91166 / 91366]*2
3. Cryotop (1 Cryotop is for maximum 4 oocytes/embryos)
4. Oocyte Cryo Plate [Ref. 83061] (for oocytes) / Repro Plate (for embryos)
5. Pipette for handling oocytes / embryos (hereinafter referred to as “pipette”)
6. Timer (with count up function)
7. Liquid nitrogen
8. Cooling Rack [Ref. 84014] for liquid nitrogen container
9. Forceps
10. Micro pipettes: 20µL (for oocytes), 300µL
11. Cryo storage equipment (cane, etc.)
12. Stereo microscope (stage must be turned off)

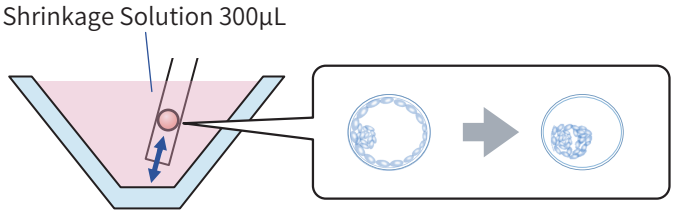
*1 For oocytes vitrification
*2 For preparation of blastocysts vitrification

TIPS

- Use pipette that has a suitable internal diameter for oocytes or embryos for better handling. (For oocytes: about 120-140µm, for cleavage stage embryos: about 150-180µm ,and 180-250µm for blastocysts.)
- All vitrification procedures are performed at room temperature.

PART2 Preparation before Blastocysts Vitrification

It is reported that embryos with a zona pellucida inner diameter >140 µm are more likely to have insufficient replacement of water and cryoprotectants, and that artificial shrinkage before vitrification improves implantation, pregnancy, and birth rates.
We recommend performing artificial shrinkage with Shrinkage Solution VT505-4 / VT525-4 before starting ES equilibration.
※J Assist Reprod Genet（2016）33:461–466



Preparation

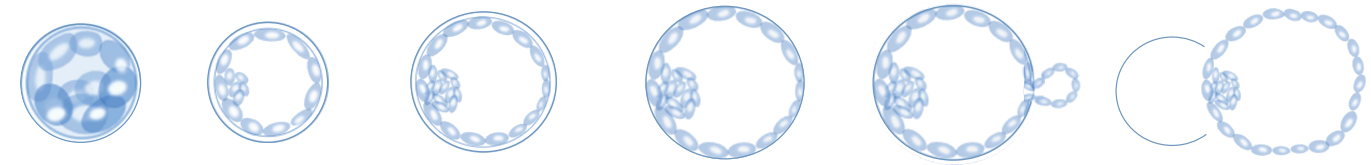
Bring Shrinkage Solution to room temperature and dispense 300 µL on Repro Plate, cover with a lid and leave it before use.
ES equilibration procedure will follow when shrinkage is confirmed, so preparation for ES equilibration on P.5 (Prepare one ES well and two VS wells, respectively 300µL) should be made at the same time.

STEP1 Blastocysts Shrinkage

Transfer the blastocysts into the Shrinkage Solution and conduct pipetting to stimulate shrinkage .
After confirming the shrinkage of the blastocysts, accelerate ES equilibration on P.5.
※ Proceed to ES equilibration after 2 minutes if the shrinkage of the blastocysts cannot be confirmed.

Information

Applicability Guide for Shrinkage Solution



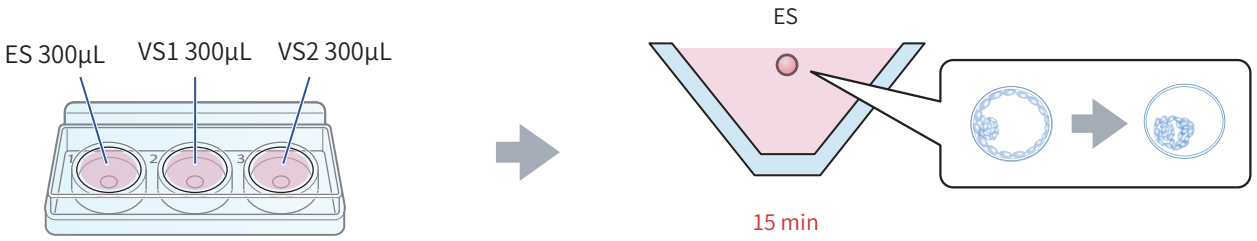
early blastocyst | full blastocyst | expanding blastocyst Gardner Grade 3 | expanded blastocyst Gardner Grade 4 | hatching blastocyst | hatched blastocyst

Shrinkage with a Shrinkage Solution (VT505-4 / VT525-4) is recommended from and after the expanding blastocyst stage

PART3 ES Equilibration

ES equilibration procedure is different for oocytes and embryos. Refer to this page for embryos and P.6 for oocytes. (The subsequent procedures are the same between the two.)

Embryos ES Equilibration



Preparation

Prepare one ES well and two VS wells, respectively 300µL on a Repro Plate.
Cover the Repro Plate until ready to use.

STEP1 Transfer of Embryos to ES

Aspirate the embryos near the tip of the pipette and gently place it with minimal volume of culture medium to the surface center of ES to avoid dilution.

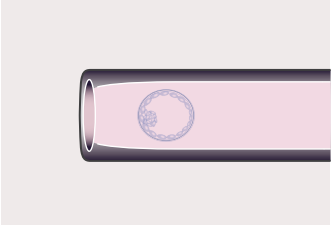


Fig.1

STEP2 ES Equilibration

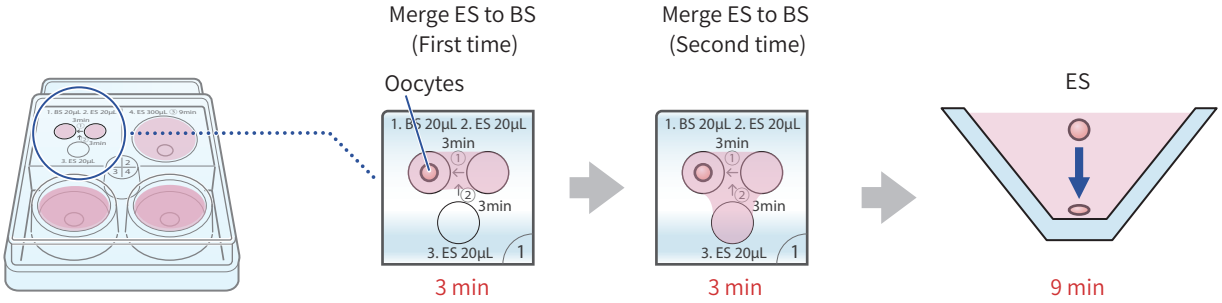
Set the timer and leave the embryos still for 15 min.
The embryos placed on the surface will sink as they shrink in about 30 seconds.

to P.7

Vitrification Protocol

The ES equilibration procedure is different for oocytes and embryos. Refer to this page for oocytes and P.5 for embryos. (The subsequent procedures are the same between the two.)

Oocytes ES Equilibration



Preparation

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).

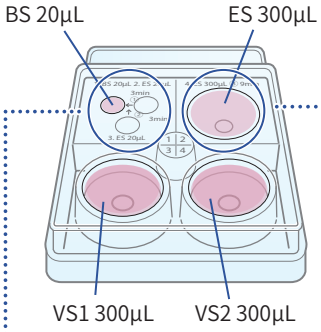


Fig.1

STEP1 Transfer of Oocytes to BS

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

STEP2 Merging ES (First time)

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).

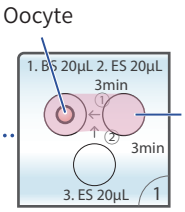


Fig.2

STEP3 Merging ES (Second time)

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).

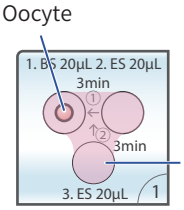


Fig.3

STEP4 Transfer of Oocytes to ES

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

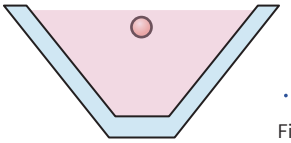


Fig.4

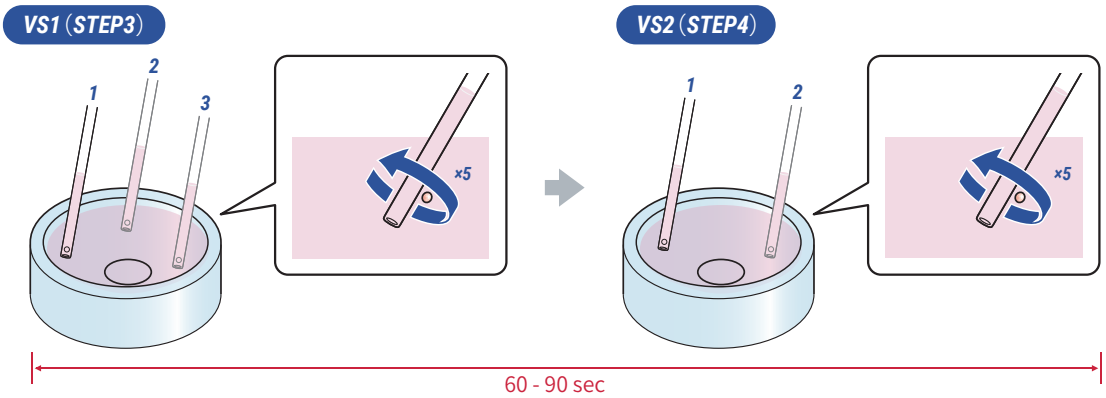
9 min.

TIP

- Although it may be difficult to confirm the recovery of oocytes depending on the angle of observation, STEP4 is completed within 9 minutes even in such cases.
- Merging the drops allows gentle osmolality change, so leave the oocytes untouched.

PART4 VS Equilibration

From this page onwards, the procedures for oocytes and embryos are the same.
Prepare Cryotop and liquid nitrogen before starting the following steps.



STEP1 Transfer of Oocytes/Embryos to VS1

After completion of ES equilibration, aspirate the oocytes/embryos with minimal volume of ES at the tip of the pipette (Fig. 1).
Transfer the aspirated oocytes /embryos to the surface of VS1 and set the timer.

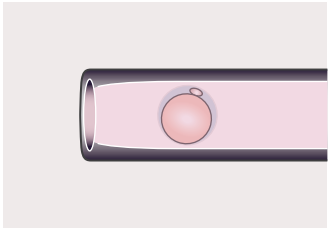


Fig.1

STEP2 Washing Pipette

Expel the remaining ES in the pipette to the outside the well, and wash the pipette by aspirating and expelling sufficient volume of VS (Fig. 2 and 3).

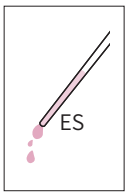


Fig.2

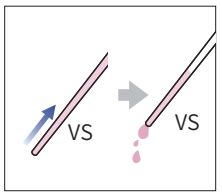


Fig.3

STEP3 VS1 Equilibration

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.

STEP4 VS2 Equilibration

Wash the pipette with VS2 as explained in STEP2 (Fig. 3).
Perform ① and ② of STEP3 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.

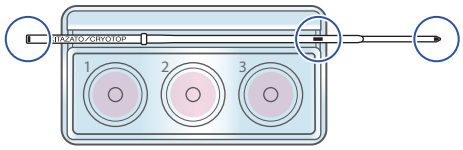
TIPS

- VS equilibration is performed efficiently by washing the pipette well with VS.
- 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.

PART5 Vitrification • Cryopreservation

Preparation

Prepare Cryotop with its black identification mark facing up.
Fill up the container with liquid nitrogen as close to its maximum capacity as possible. This is preparation to quickly plunge Cryotop into it.



STEP1 Loading Oocytes/Embryos on Cryotop

Focus on the black tip mark of Cryotop (Fig. 1), expel the VS horizontally near the black mark, and place the oocytes/embryoed (Fig. 2).

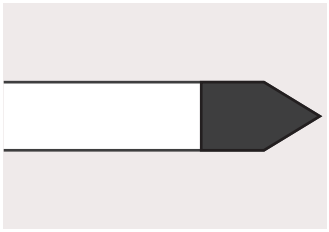


Fig.1

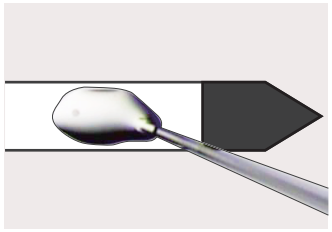


Fig.2

STEP2 Removal of Excess VS on Cryotop

Aspirate excess VS on the Cryotop from the extended drop edge, preferably from distal point from the oocytes or embryos (Fig. 3). The optimal liquid volume is such that the drop shadow is minimized or disappeared(Fig. 4).

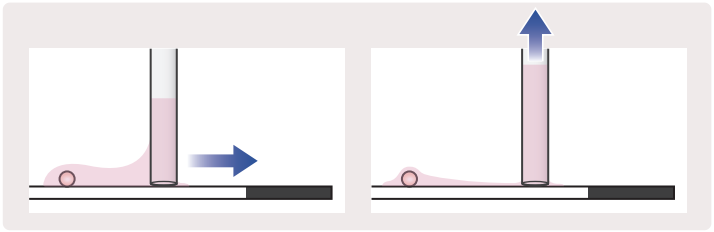


Fig.3

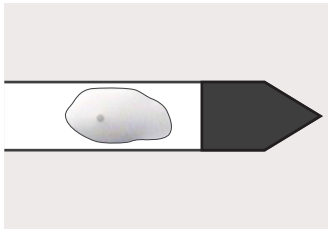


Fig.4

TIPS

- When aspirating oocytes or embryos from the VS2, make sure to hold them at the tip of the pipette.
- Make a droplet on the Cryotop by spreading the VS sideways. It prevents you from aspirating oocytes/embryos while removing excel VS.

STEP3 Confirmation of Oocytes/Embryos

Remove the pipette from the Cryotop and confirm if the oocytes/embryos are on the Cryotop.

STEP4 Rapid Cooling / Vitrification

Plunge the Cryotop into liquid nitrogen within 1 second for vitrification.

STEP5 Capping Cryotop

Keep the tip of the Cryotop in liquid nitrogen to cap it with the cover straw using forceps (Fig. 1).
Use your fingers to hold the upper part of the cover straw and twist to cap it tight in air (Fig. 2 and 3).
Tip of the Cryotop must be kept submerged in liquid nitrogen .

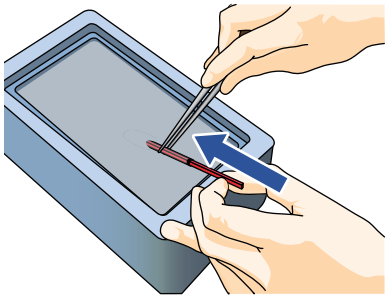


Fig.1

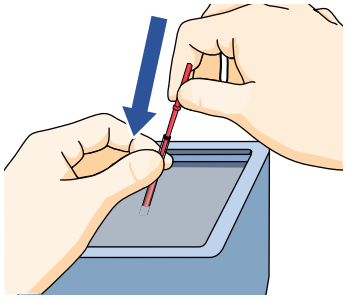


Fig.2

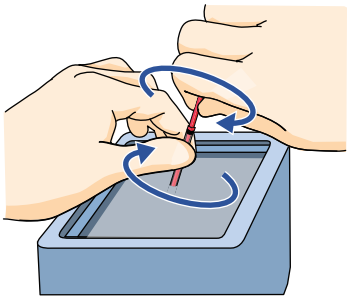


Fig.3

STEP6

Make sure the Cryotop is securely capped. Use cane to store in a liquid nitrogen tank.

TIP

Fill up the container with liquid nitrogen to enhance rapid temperature transition of Cryotop.

Information

Number of Oocytes/Embryos for one piece of Cryotop

Oocytes ：Maximum 4 per Cryotop.
Embryos：Depends on the number of embryos to be used after warming.
For example, if single embryo transfer is planned, store one embryo on one piece of Cryotop.

Warmining Protocol

Warmining Procedure

Oocytes/ Embryos → Preparation → Immersion in TS → Immersion in DS → Immersion in WS → Washing

PART 1 Materials Required

1. Thawing Media VT602 / VT622 [Ref. 91121 / 91208]
   No.1 TS (Thawing Solution): 4mL vial x 2
   No.2 DS (Diluent Solution): 4mL vial x 1
   No.3 WS (Washing Solution): 4mL vial x 1
2. 35mm dish
   ※Prewarm TS and 35mm dish to 37°C and equilibrate DS and WS to room temperature before use.
3. Repro Plate
4. Pipette for handling oocytes / embryos (hereinafter referred to as “pipette”)
5. Timer (with count up function)
6. Liquid nitrogen
7. Cooling Rack [Ref. 84014] for liquid nitrogen container
8. Forceps
9. Micro pipette: 300µL
10. Stereo microscope (Stage must be turned off).

CAUTION

- For optimal handling, please use a pipette with an appropriate internal diameter for oocytes (approximately 140-180µm), or embryos (approximately 180-250µm).
- All the warming procedures except for immersion in TS are performed at room temperature.

PART 2 Preparation

STEP 1 Warming TS

Prepare Thawing Media (VT602 / VT622).
Prewarm the TS vials capped and 35mm dishes to 37°C for the number of patients.
Required time: >30 min in an incubator and >5 min in a water bath (40°C). Do not warm more than 8 hours to maintain the quality of the product.

STEP2 Preparation of Cryotop

Remove the Cryotop from the storage tank and quickly transfer it to a container filled with liquid nitrogen.
Remove the cover straw keeping the Cryotop tip submerged in liquid nitrogen.
Place the Cryotop upright in the corner to keep the closest proximity to the TS (Fig. 1 and 2).

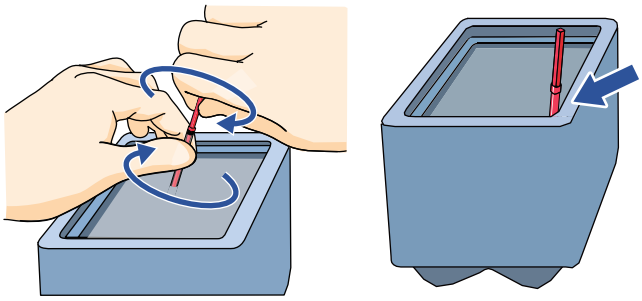


Fig.1

TIP

Fill up the container with liquid nitrogen, so that Cryotop can be transferred into TS in a short distance for warming.

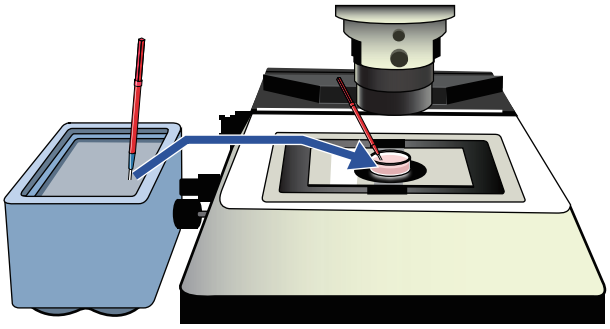


Fig.2

STEP3 Preparation of the Dish

Prepare one DS well and two WS wells on Repro Plate, respectively 300µL and cover with a lid.
After preparing the DS and WS, take out the warmed TS vial and 35 mm dish from the incubator.
Pour the entire volume into the dish (Fig. 3).

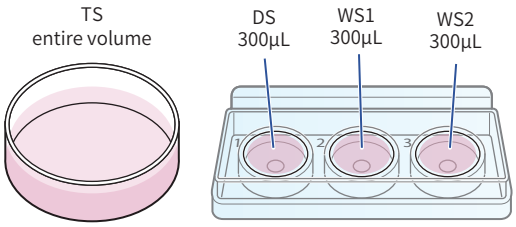


Fig.3

TIP

Invert the TS vial five times to mix its contents for its high concentration of cryoprotectants. The entire volume of TS must be prepared in the dish.

STEP4 Setting of a Stereo Microscope

Focus on the center of the TS using the pipette in advance (Fig. 4), and set the magnification of the microscope to the minimum (widest field of view).

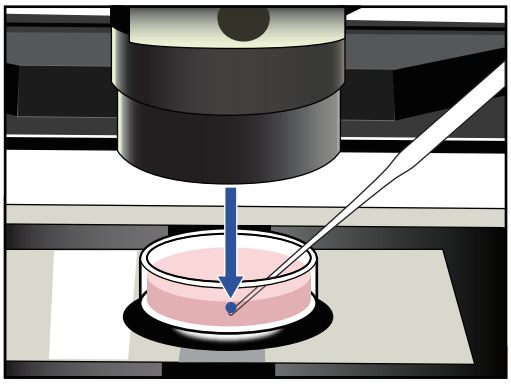
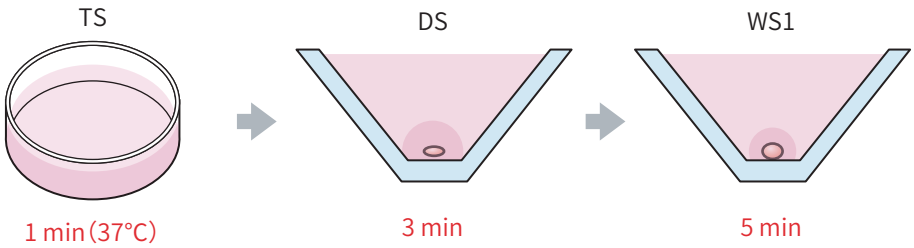


Fig.4

PART3 Immersion in TS, DS and WS1



STEP1 Immersion in TS

Start the timer. (It is easier to check time with a count-up function).
Within 1 second, immerse the Cryotop sheet into TS.
Adjust the microscope focus on the tip of the sheet for oocytes/embryos. Continue observing even after the oocytes/embryos naturally detach themselves from the sheet. Don't touch it for one minute.

TIP

If the oocytes / embryos remain attached to the sheet after 1 minute in TS, gently expel some of the TS over them or give vibration to the cryotop by flicking its tip with a pipette, and let them detach. In either method, proceed with care to avoid damaging the oocytes / embryos, regardless of the elapsed time in TS.

STEP2 Immersion in DS

After immersion in TS, aspirate the oocytes/embryos and additional TS for approximately 2 mm with the pipette (Fig. 1).
Put the pipette tip in the center of the DS well bottom and place the oocytes/embryos in the TS layer expelled in the DS. Leave it for 3 min (Fig.2).

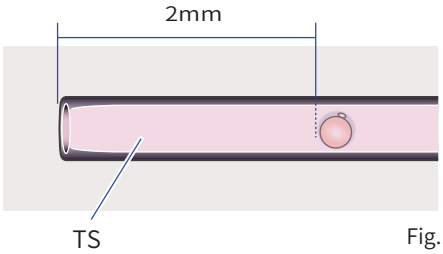


Fig.1

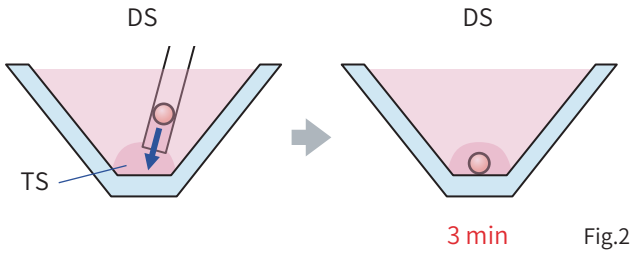


Fig.2

STEP3 Immersion in WS1

Wash the pipette with DS.
Aspirate the oocytes/embryos and additional DS for approximately 2 mm with the pipette. Put the pipette tip in the WS1 well bottom and place the oocytes/embryos in the DS layer expelled in the WS1. Leave it for 5min.

TIPS

- Gradual osmolality change is important in the dilution process to ensure the previous solution mix gently with a new one.
- The “2mm” aspiration can be approximate. The purpose here is to bring in the previous solution to the next. The width of Cryotop sheet is 1mm, hence a guide when making a 2mm guesstimate.

PART4 Washing

STEP1 Washing in WS2

Wash the pipette with WS2.
Aspirate the oocytes/embryos at the tip of the pipette (Fig. 1) and transfer them to the surface of WS2. It is to minimize the volume of the WS1 carried into the WS2.
After the oocytes / embryos settle to the bottom, aspirate and transfer them to the surface of the WS2 and wait for them to settle to the bottom (Fig. 2). No need to check the time here.

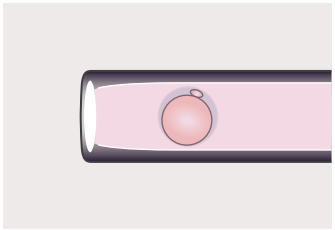
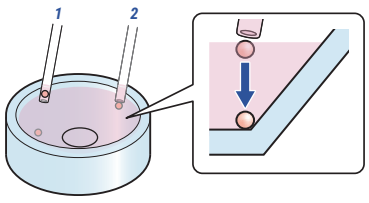


Fig.1



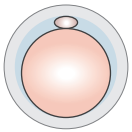
WS2

Fig.2

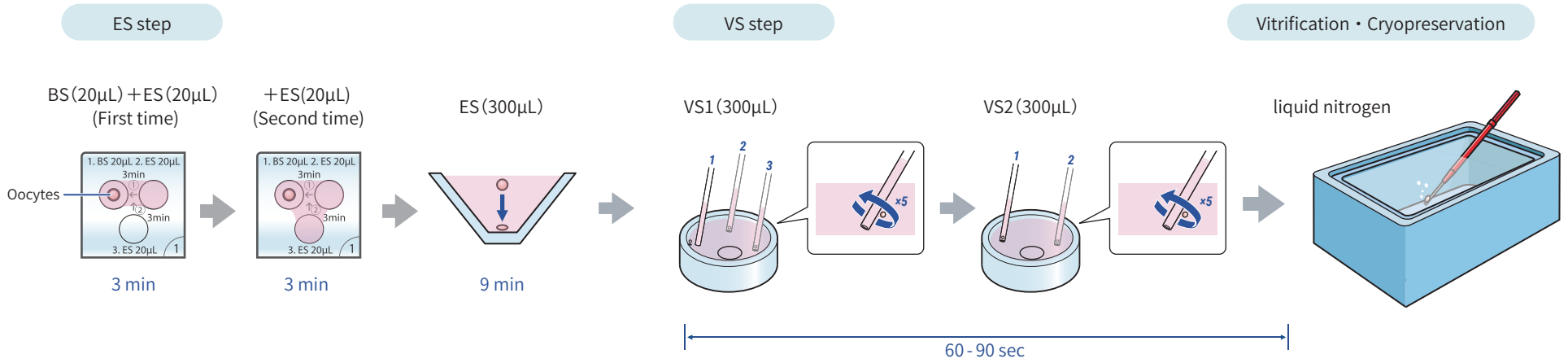
STEP2 Recovery Culture

Perform recovery culture of oocytes/embryos after washing.
The estimated recovery culture time is as below:
Oocytes: 2 to 3 hours
Embryos: 30 min to 2 hours
The recovery culture for embryos is performed to confirm the embryos's viability. Once survival is confirmed, proceed to the next procedure regardless of time. It takes 2 to 3 hours for oocytes spindles to recover. Start ICSI or other following procedures after confirming recovery of spindle or after 2 to 3 hours of recovery culture.

Oocytes Protocol

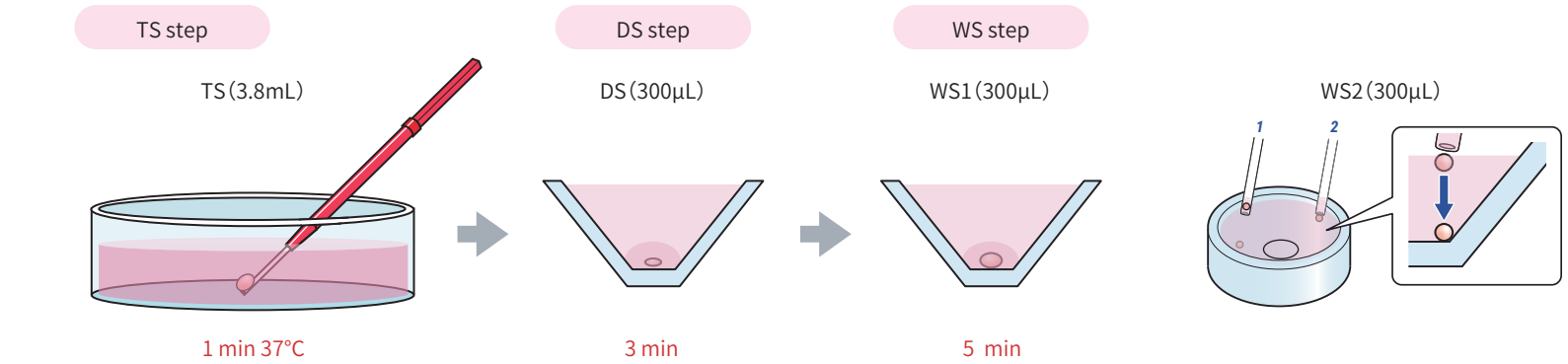


Vitrification



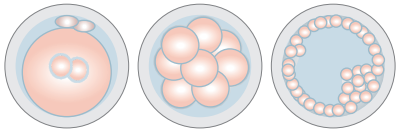
Room temperature

Warmining

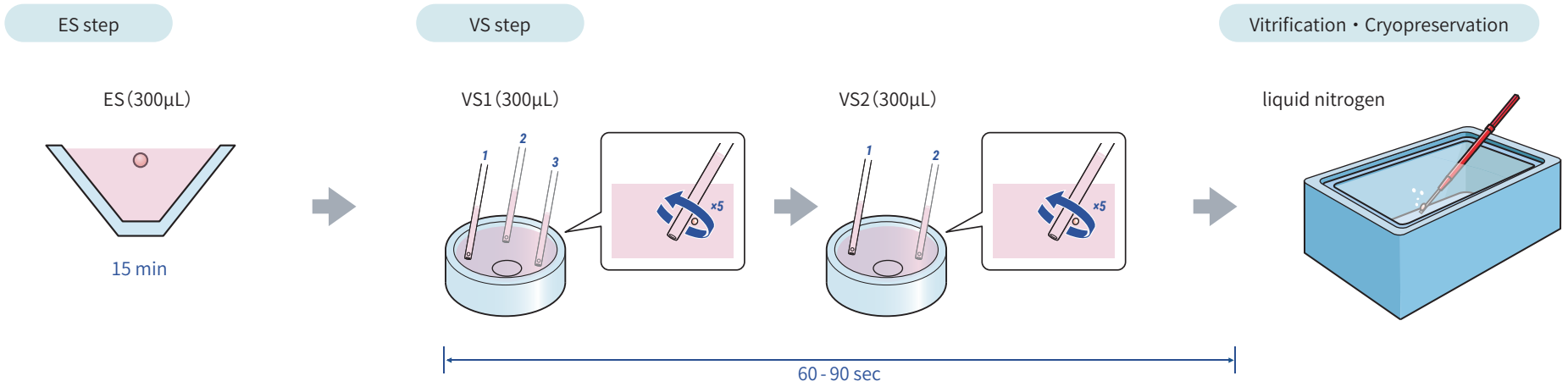


37°C | Room temperature

Embryos Protocol

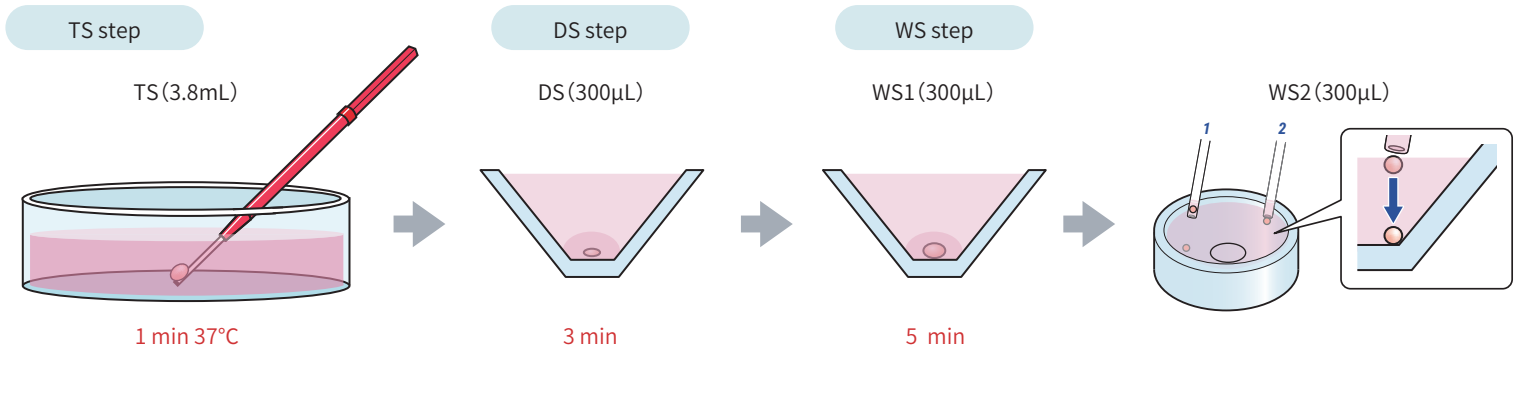


Vitrification



Room temperature

Warmining



37°C | Room temperature

Quality Results for Life

2023.11.20 ver.3.00

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