OVA CRYO KIT

Protocol

Ovarian tissue
Vitirification Method

KITAZATO®

Vitrification

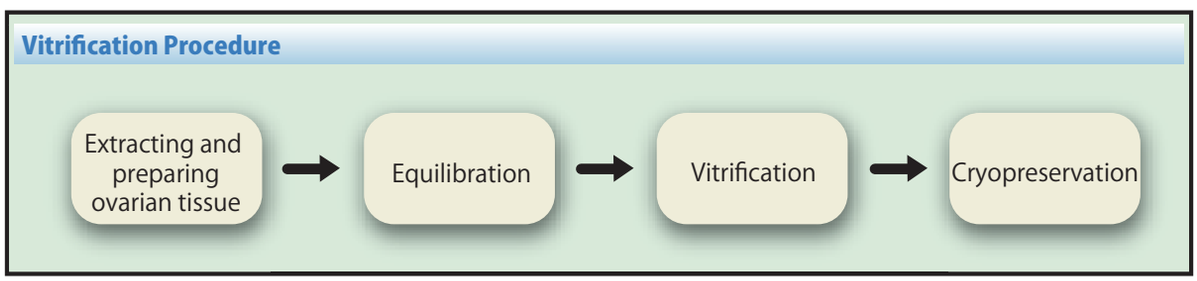
Products Used

Ova Cryo Kit (Ref. 82212 Code. VT301S)

- Cryo 1: 20 mL x 1
- Cryo 2: 20 mL x 1
- Cryo 3: 20 mL x 1

Ova Cryo Device Type M (Ref. 81213 Code. ODT x 10) : for open system

Ova Cryo Closed Device CryoSheet (Ref. 81214 Code. OCS10) : for closed system



Materials Required

- Ova Cryo Kit (Ref. 82212 Code. VT301S)
- Ova Rinse (Ref. 82215 Code. OVR-100)
- Dish (OD 60mm) x 3 : for Cryo1,2,3
- Dish (OD 60mm - 100mm) x 2 : for immersing the ovary in Ova Rinse
- 1mm syringe and 21G injection needle x 5 sets
- Scissors
  Note : 2 types of scissors; straight-end and curved-end facilitate preparation of ovarian tissue
- Curved tweezers (about 12cm)
- Tweezers (about 20cm) x 2
- Surgical knife (Blade No. 10)
- Sterilized gauze
- Sterilized drape : to cover clean bench
- Timer
- Liquid nitrogen with its container (e.g. styrofoam)
- Cane (C-2 Cane)

【 For alternative method 】

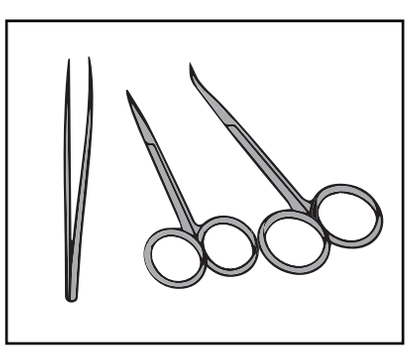
- Square Measure
- Microtome blade with handle
- Surgical knife(Blade No.11)

【 For open system 】

- Ova Cryo Device Type M (Ref. 81213 Code. ODT x 10)

【 For closed system 】

- Ova Cryo Closed Device CryoSheet (Ref. 81214 Code. OCS x 10)
- Sealer
- CryoSheet Cane (Ref. 81215 Code. CryoSheet Cane x 10)



STEP 1 Preparation

- Bring Cryo1, Cryo2 and Cryo3 to room temperature (25 - 27℃).
- Label the dishes “Cryo1” , “Cryo2” and “Cryo3”. Put full content of the media in the respective dishes.

STEP 2 Preparing Ovary into Slices

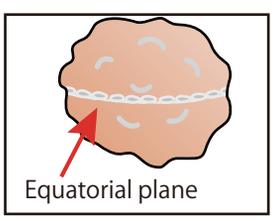
1. Wash an extracted ovary with saline and remove extra blood.
2. Puncture follicles with injection needle and aspirate the fluid with syringe.

Note: For the collected immature oocytes, culture for IVM and then vitrify the matured oocytes.
For the collected mature oocytes, vitrify or culture them in vitro.

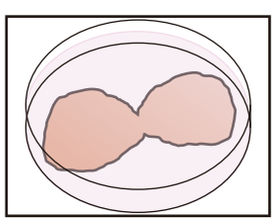
Method 1

For the following procedures, be careful not to dry the ovary and use Ova Rinse (Ref. 82215 Code. OVR-100).

1. Cut the ovary with scissors along the equatorial plane. Once you make a small cut at the equatorial plane, insert the tip of the scissors vertically into the medulla and spread them. Repeat this all along the equatorial plane.

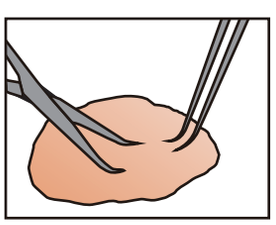


2. Open the ovary in two pieces along the equatorial plane.
For the following procedure, start with one half first and leave the other in Ova Rinse.



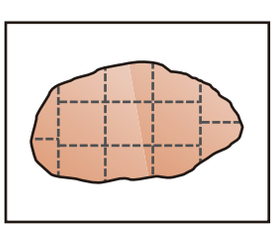
3. Medulla should be removed and only cortex is to be used for vitrification. Gently hold the ovary with tweezers and cut off the medulla with scissors until only 1mm thin cortex is left. This process should be done in Ova Rinse.

Note : Scissors with curved-end facilitate removing medulla.
Note : Image of cutting off medulla is like lifting medulla as you cut it.
Do not rush and take time in this process, cutting it little by little.



4. Once you have cortex without medulla, use surgical knife and cut it into 1cm x 1cm size.

Notes : Remaining medulla at the rim of ovary can be removed after ovary is cut in pieces.

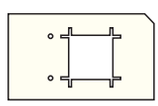


STEP 2 Preparing Ovary into Slices

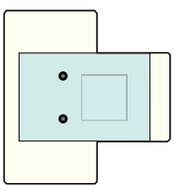
Method 2 For the following procedures, be careful not to dry the ovary and use Ova Rinse (Ref. 82215 Code. OVR-100).

Method 1 is recommended, but if you have difficulty, Square Measure can be used alternatively. It is an easier procedure, but disadvantage is that final number of ovarian tissue and oocytes collected from Ova Rinse may be reduced.

Square Measure is composed of 2 parts; A and B.



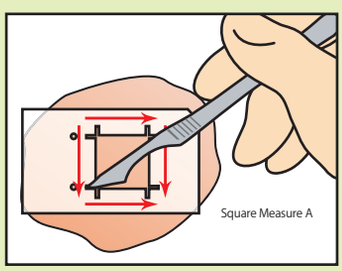
Square Measure A

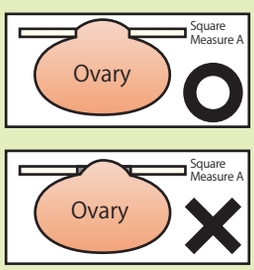


Square Measure B

1. Wipe the surface of ovary with sterilized guaze to prevent Square Measure from slipping on the its surface.
2. Place Square Measure A on the surface of the ovary with its metal part on the left.
3. Cut ovary along the inner edge of Square Measure A.

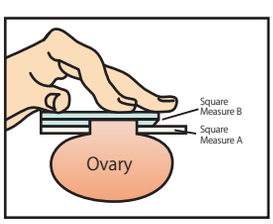
TIP



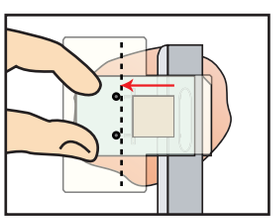


Be sure to cut ovary from inner edge of Square Measure A .
(cut by drawing Sharp "#" line)

4. Attach Square Measure B to Square Measure A and press it on the ovary surface. Insert Microtome between Square Measure A and B, and slice the tissue until it reaches the metal stopper.

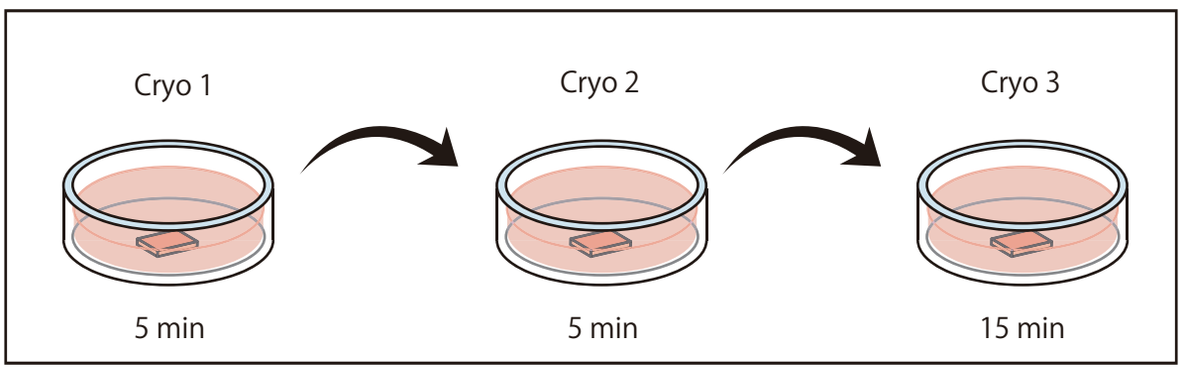


5. Detach the Square Measure and transfer the ovarian tissue into Ova Rinse to prevent it from drying.



6. Use scissors to cut off the remaining medulla until you get equel thickness of 1mm.

STEP 3 Equilibration of Ovarian Tissue



Multiple ovarian tissues can be processed together in Cryo1, 2 and 3.

1. Wipe the ovarian tissue with gauze and transfer the tissue into Cryo1. Let the tissue remain in Cryo1 for 5 min.

2. Take the tissue in Cryo1, remove excess medium at the brim of the dish, and transfer it into Cryo2. Let the tissue remain in the Cryo2 for 5 min.

3. Take the tissue in Cryo2, remove excess medium at the brim of the dish, and transfer the tissue into Cryo3. Let the tissue remain in Cryo3 for 15 min.

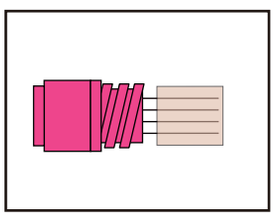
4. Prepare ODT and liquid nitrogen while you wait for equilibration of ovarian tissue.

STEP 4 Vitrification of Ovarian Tissue

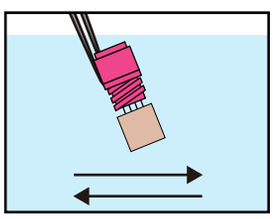
Open System

1. Prepare the number of ODT that matches the number of ovarian tissue prepared. Mark all ODT vials with patient ID and neccessary information.

2. Wipe the ovarian tissue with gauze. Place the ovarian tissue on ODT spreading and maximizing its surface area. The ovarian tissue should be placed more to the metal tip to avoid placing it next to the cap part where metals are embedded. Leave 5mm space open from the cap part.



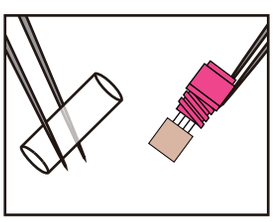
3. Hold the cap of ODT with tweezers and plunge into liquid nitrogen.



Note : Plunge ODT into liquid nitrogen from the surface ovarian tissue is placed on. This will avoid the tissue from coming off ODT.

Note : In case ovarian tissue came off ODT, place the ovarian tissue in the vial first, then close the cap.

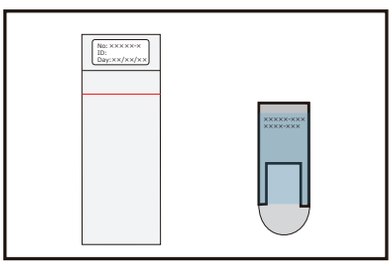
Note: After plunging ODT into liquid nitrogen, shake it about, removing the surrounding air.



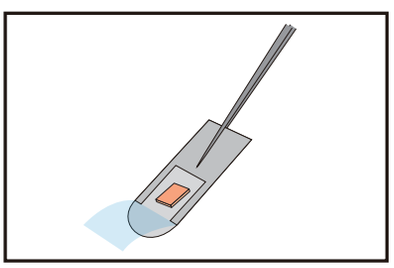
STEP 4 Vitrification of Ovarian Tissue

Closed System

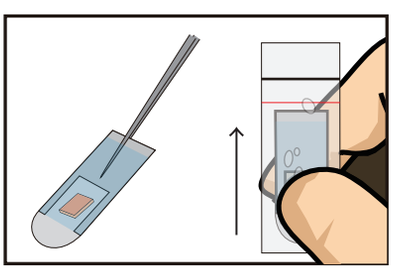
1. Prepare the number of CryoSheet with attached pouch that matches the number of ovarian tissue prepared. Mark all CryoSheet and pouches with patient ID and necessary information.



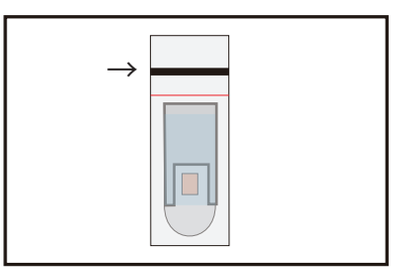
2. Wipe the ovarian tissue with gauze. Flip the film of the CryoSheet and place the ovarian tissue on it, spreading and maximizing its surface area.



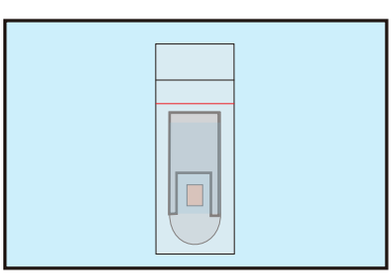
3. Flip back the film and push out the air, using your finger or tweezers. Make sure the ovarian tissue is appressed against the film. Put the CryoSheet into the pouch and push out the air as well.



4. Seal the pouch at the black line with the heat sealer.



5. Hold the CryoSheet with a tweezer, and plunge it into the liquid nitrogen.



Note: Using the sealer with inappropriate temperature may cause the pouch to be ripped. Please use the sealer with the applicable temperature.

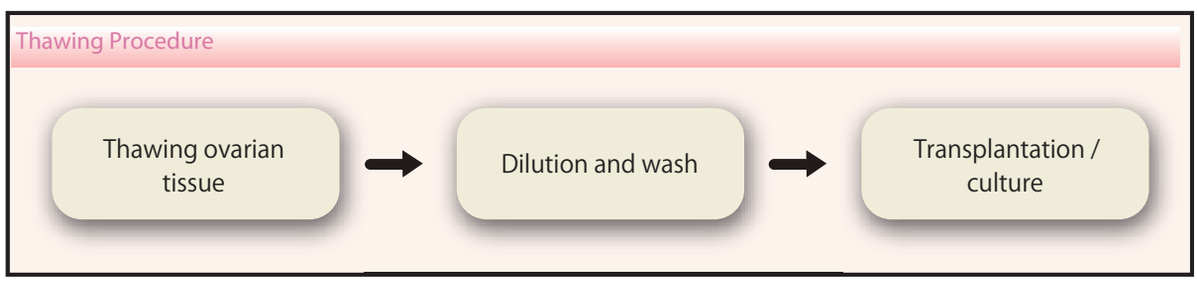
Note: After plunging CryoSheet into the liquid nitrogen, shake it about gently, removing the surrounding air.

Thawing

Products Used

Ova Thawing Kit (Ref. 82222 Code. VT302S)

- Thaw 1: 100 mL x 1
- Thaw 2: 20 mL x 1
- Thaw 3: 20 mL x 1



Materials Required

- Ova Cryo Kit (Ref. 8222 Code. VT302S)
- Liquid nitrogen with its container (e.g. styrofoam)
- Water bath
- Dish (OD 60mm - 100mm) x 2
- Container (110mL / 4.5 oz.)
- Tweezers (about 12cm) : for dilution and washing of ovarian tissue
- Tweezers (about 20cm) : for thawing procedure
- Timer
- Ova Culture (Ref.82216 Code.OVCL-100) or Ova Culture with HEPES (Ref.82217 Code. OVCM-100)
- Sterilized drape : to cover clean bench

【For Closed system】

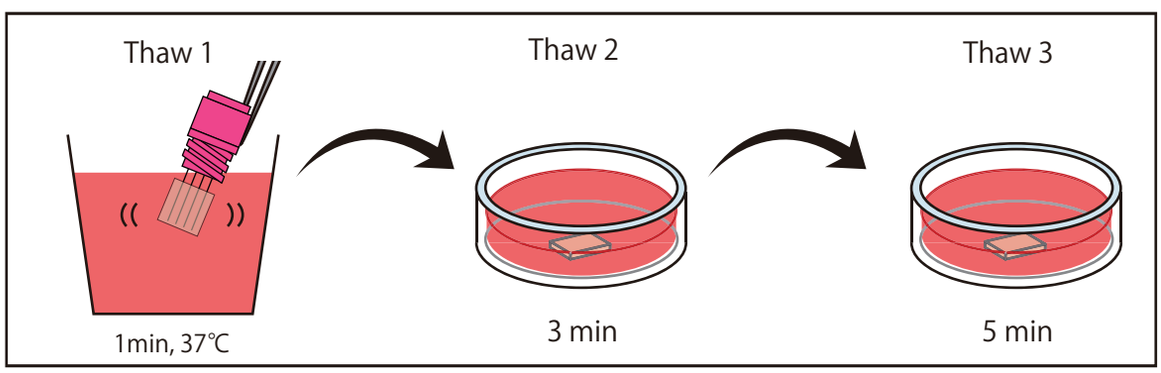
- Scissor : for cutting the pouch

STEP 1 Preparation

1. Put full content of Thaw1 in the container and warm it to 37℃ in a water bath.
2. Bring Thaw 2 and Thaw 3 to room temperature (25 - 27℃).
3. Label dishes “Thaw 2” and “Thaw 3”. Put full content of the media in the respective dishes.
4. Prepare liquid nitrogen next to the water bath. This is to speed up warming by minimizing the transferring time in air.

STEP 2 Thawing

Open System



1. Open ODT in liquid nitrogen with tweezers and remove the vial. Confirm the ovarian tissue on ODT cap.
2. Plunge ODT into Thaw 1 warmed to 37℃ in the water bath. Shake ODT in Thaw1 to detach the ovarian tissue and immediately remove ODT from Thaw 1. The ovarian tissue should remain in Thaw 1 for 1 min.

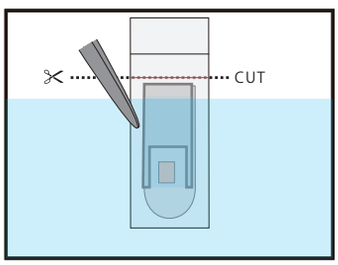
   Note : Take care to dip only the metal part of ODT into Thaw 1 to maintain the warm temperature.
3. Transfer the tissue from Thaw 1 into Thaw 2 at room temperature. Leave it in Thaw 2 for 3 min.
4. Transfer the tissue from Thaw 2 into Thaw 3 and leave it for 5 min.

   After 5 min in Thaw3, culture the tissue for recovery before transplantion. When implanting immediately after thaw, culture in OVCM-100 for 30 min. If you have longer time for recovery culture, use OVCL-100 in an incubator.

STEP 2 Thawing

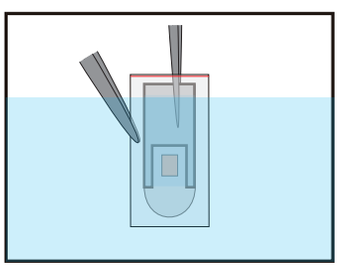
Closed System

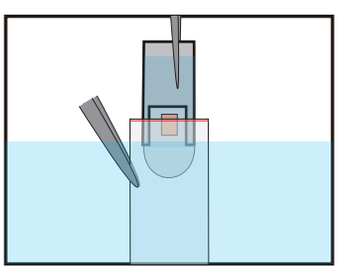
1. In the liquid nitrogen, cut the sealed pouch at the red line.

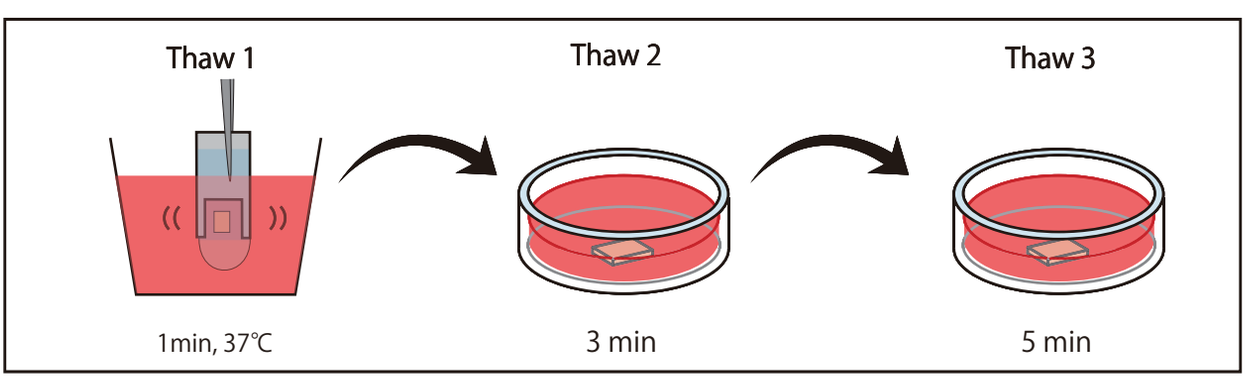


Note: Avoid the CryoSheet having direct contact with the liquid nitrogen.
Do not immerse the whole pouch into the liquid nitrogen
nor tilt the pouch in an angle which the liquid nitrogen might get in.

2. Take out the CryoSheet from the pouch and transfer it into Thaw1 brought to 37℃ in a water bath.







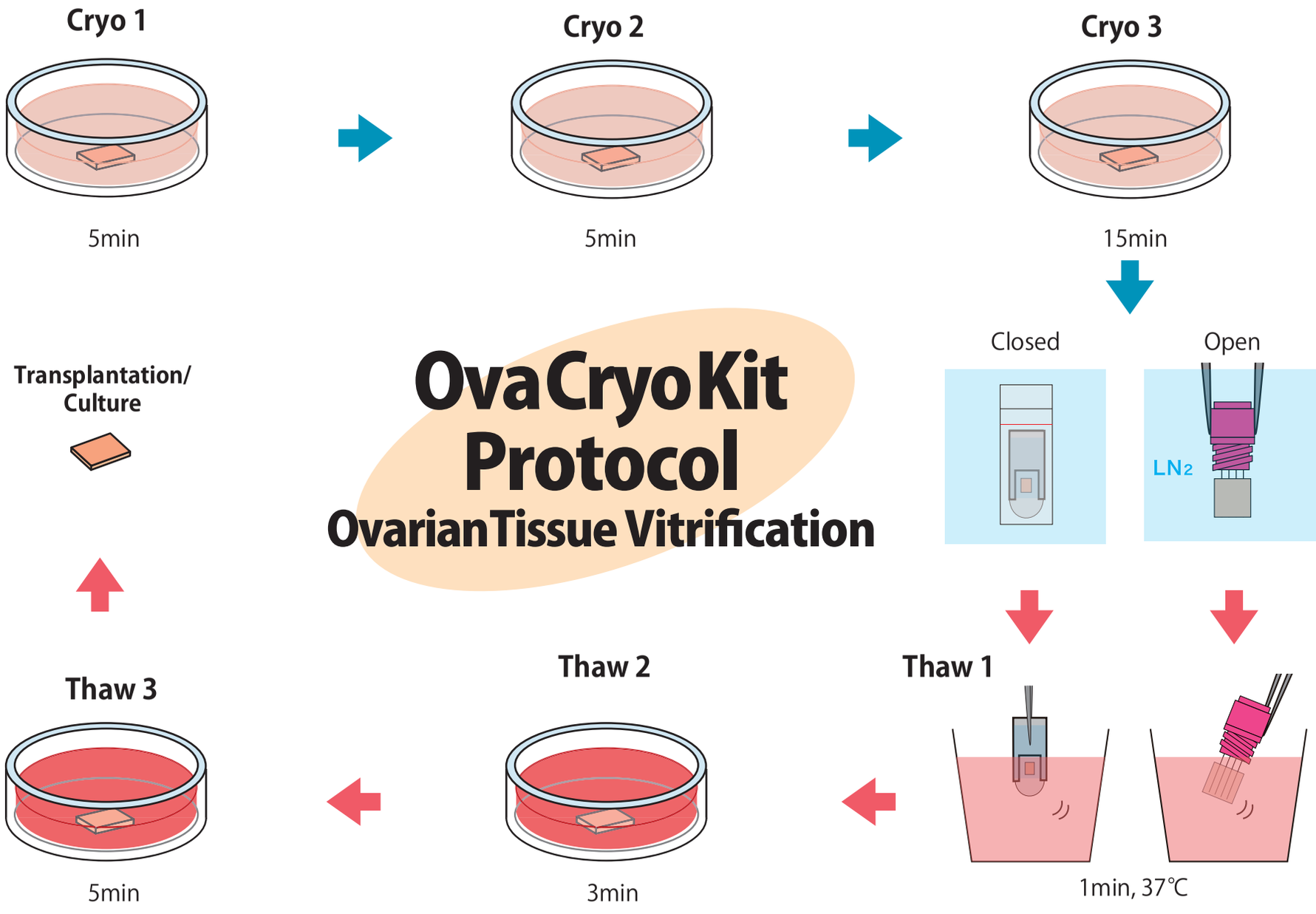
3. Transfer the tissue from Thaw 1 into Thaw 2 at room temperature.
Leave it in Thaw 2 for 3 min.

4. Transfer the tissue from Thaw 2 into Thaw 3 and leave it for 5 min.

After 5 min in Thaw3, culture the tissue for recovery before transplantion. When implanting immediately after thaw, culture in OVCM-100 for 30 min. If you have longer time for recovery culture, use OVCL-100 in an incubator.

Ova Cryo Kit Protocol

Ovarian Tissue Vitrification



KITAZATO®

www.kitazato.co.jp

Jun 1, 2021 Ver.2