SIMPLIFIED WARMING PROTOCOL FOR CRYOLOCK®

Oocytes and Embryos

STEPS 9-12 MUST BE PERFORMED AT ROOM TEMPERATURE (22 - 27°C)

Do not begin warming procedure until you have a pre-equilibrated dish of appropriate culture medium supplemented with SSS or DSS at 20% (v/v), or HSA at 12 mg/ml for final recovery of specimen(s).

Have all necessary materials, tools and equipment ready and easily accessible before starting procedure. Refer to Cryolock warming protocol and product insert for detailed instructions and warnings.

1. Set up thawing dishes (as shown in diagram):
   - **At 37°C:** Aseptically dispense a minimum volume of 1 mL of TS and warm to 37°C in an incubator without CO_2 or on a heating stage at least 30 minutes prior to starting warming procedure.
2. Identify the Cryolock sample(s) to be warmed and quickly transfer from LN_2 storage to an LN_2 filled holding reservoir in preparation for warming procedure.
3. Place LN_2 filled holding reservoir in close proximity to the working area and stage of the microscope in order to achieve subsequent rapid manipulation from reservoir to TS.
4. Remove TS dish from 37°C incubator or heating stage and place it under focus on top of the microscope stage.
5. Using forceps, hold the upper end of the Cryolock body with the identification label facing up.
   - Option A*: Quickly but gently remove the cap under LN_2, twisting the parts until release.
   - Option B: Quickly take the Cryolock out from the LN_2, then quickly remove the cap with a gentle twist.

   NOTE: Laboratory should consult their own procedures and protocols.
6. Immediately plunge the concave tip of the Cryolock, with the specimen(s) facing up, into the 37°C TS. Under microscopic observation, gently move the Cryolock until the specimen(s) are released from the tip.
7. Leave the specimen(s) for a total of 1 minute in the TS.
8. Thirty (30) seconds following the initial plunge, gently pipette the specimen(s), if floating, and place at the bottom of the TS.

Steps 9-12 must be performed at room temperature (22-27°C).

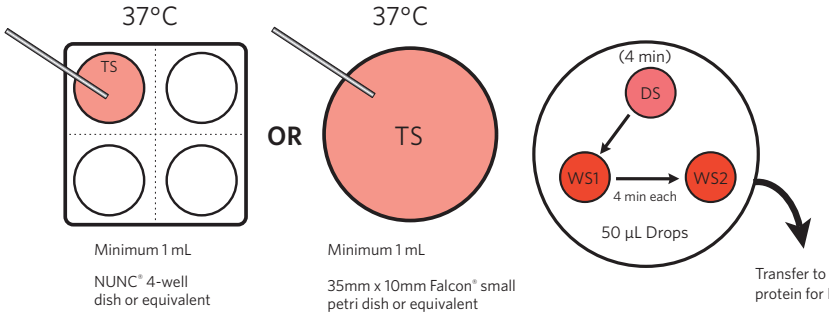
- **At room temperature:** Aseptically dispense one (1) 50 µL drop of DS on a sterile Petri dish

9. Transfer specimen(s) to DS for 4 minutes. Gently pipette specimens once to ensure complete rinse in DS.
10. During the 4 minute exposure in DS, aseptically dispense two (2) 50 µL drops of WS (WS1, WS2) as shown in diagram.
11. Transfer specimen(s) to WS1 then WS2 for 4 minutes each, undisturbed.
12. Transfer warmed OOCYTE(S) to pre-equilibrated culture medium with 20% (v/v) protein supplement or 12 mg/mL for recovery (2-3 hours to allow time for spindle re-formation) prior to subsequent manipulations.

    There are two options for warmed EMBRYO(S):

    a) For immediate transfer to patient: transfer EMBRYO(S) to pre-equilibrated 'transfer' medium containing 20% (v/v) protein supplement or 12 mg/mL.

    b) For further culture: transfer EMBRYO(S) to pre-equilibrated culture medium containing 20% (v/v) protein supplement or 12 mg/mL for a 4 hour recovery period. After recovery period, transfer EMBRYO(S) to culture medium with 10% (v/v) protein and incubate accordingly until desired developmental stage has been reached for transfer to patient.



KEY	
TS	Thawing Solution
DS	Dilution Solution
WS	Washing Solution
→	Transfer specimen to next drop

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*The method described in Option A is not cleared in the U.S.

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Tips

- Have all necessary materials, tools and equipment ready and easily accessible before starting procedure.
- Refer to the Cryolock Product Insert for specific instructions.
- Pre-equilibrate a dish of appropriate culture medium with 20% (v/v) or 12 mg/mL protein for final recovery of specimen(s).
- Cryolock must remain submerged in LN_2 until ready to warm. When transferring Cryolock from LN_2 filled holding reservoir, or between LN_2 storage tanks, vitrification devices should always be submerged in an LN_2 filled goblet to prevent uncontrolled/ premature warming in air.
- Set up warming dish with a minimum volume of 1 mL of TS, at least 30 minutes prior to beginning procedure.
- Remove the TS dish from 37°C incubator or heating stage right before the warming procedure.
- Immediately immerse the concave tip of the Cryolock into the 37°C TS after removed from LN_2 (within 2 seconds).
- Under microscopic observation, gently move the Cryolock until the specimen(s) are released from the tip.
- Keep focusing under the microscope while immersing tip into TS to locate the specimen(s).
- Limit exposure to light while moving the specimens through the solutions.
- If you choose to, you may overlay DS and WS drops with 8.0 mL to 8.5 mL of equilibrated mineral oil, at least 45 minutes prior to starting warming procedure.
- **NOTE:** Following complete recovery (2-4 hours post-warming), oocytes must be fertilized by ICSI due to zona hardening during vitrification.

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