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Warming Oocytes and Embryos Protocol with Vit Kit - Warm NX

The following protocol is for use of Vit Kit - Warm NX (PN 90183) with vitrification/cryostorage devices that require direct plunge/contact into Thawing Solution. Vit Kit - Warm NX contains Thawing NX - TS (TS), Dilution NX - DS (DS), and Washing NX - WS (WS).

Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure. The warming steps include plunging the device into the 37°C TS and subsequent diluting and washing in DS and WS at room temperature.

INITIAL PREPARATION

1. Set-up thawing dish (as shown in Figure 1):
   - **At 37°C:** Aseptically dispense a minimum volume of 1 mL of TS and warm to 37°C in a humidified incubator without CO_2 or on a heating stage at least 30 minutes prior to starting warming procedure.
2. Identify the device 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. Prepare the device for warming by referring to corresponding device IFU and internal laboratory procedure(s).
   - *Laboratory should consult their own procedures and protocols.*
5. Remove TS dish from 37°C incubator without CO_2 or heating stage and place it under focus on top of the microscope stage.
6. After specimen(s) are in TS following the device-specific protocol, leave the specimen(s) for a total of 1 minute.
   - Thirty (30) seconds following exposure into TS, gently pipette the specimen(s) if floating, and place at the bottom of the TS.
   - **At room temperature:** Aseptically dispense one (1) 50 µL drop of DS on a sterile Petri dish (see Figure 2).

Steps 7–10 must be performed at room temperature (20–27°C).

7. Transfer specimen(s) to DS for 4 minutes. Gently pipette specimen(s) once to ensure complete rinse in DS.
   - *The specimen(s) will remain shrunken during exposure to DS.*
8. During the 4-minute exposure in DS, aseptically dispense two (2) 50 µL drops of WS (WS1, WS2) as shown in Figure 2.
9. Transfer specimen(s) to WS1 then WS2 for 4 minutes each, undisturbed.
   - *The specimen(s) should rehydrate and reconstitute perivitelline space to the original size within 2–3 minutes in WS.*
10. Process the specimen(s) as indicated below:

    a) OOCYTE(S) should be transferred to pre-equilibrated culture medium in accordance with laboratory protocol for recovery (2–3 hours to allow time for spindle re-formation) prior to subsequent manipulations.

    b) There are two options for warmed EMBRYO(S):

    i) For immediate transfer to patient: Transfer EMBRYO(S) to pre-equilibrated transfer medium.

    ii) For further culture: Transfer EMBRYO(S) to pre-equilibrated culture medium for a 4-hour recovery period. After recovery period, transfer EMBRYO(S) to culture medium with 10% (v/v) protein (10% v/v when using Serum Substitute Supplement or Dextran Serum Supplement; 5% v/v when using Human Serum Albumin) and incubate accordingly until desired developmental stage has been reached for transfer to patient.

For additional details on the use of these products, each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

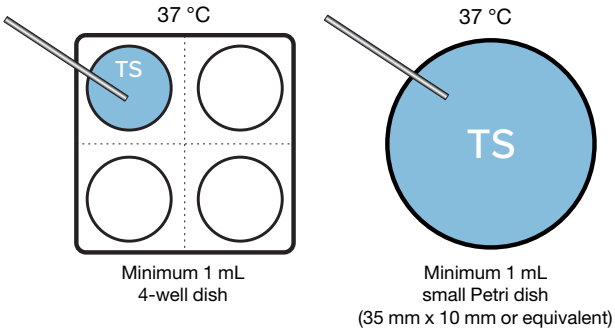

Figure 1

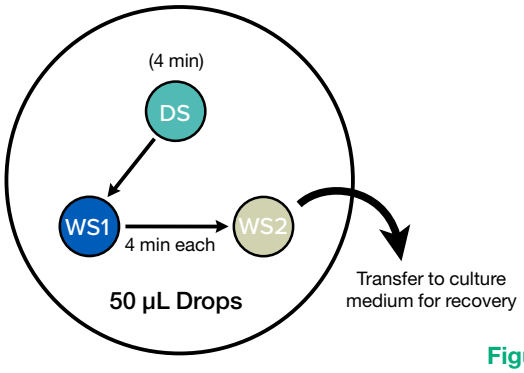

Figure 2

Warming Oocytes and Embryos Protocol with Vit Kit - Warm NX

TIPS FOR WARMING OOCYTES & EMBRYOS

- Recovery medium
  - For best results, we recommend oocytes and embryos to recover in a pre-equilibrated culture medium with 20% (v/v) or 12 mg/mL protein supplement (SSS or DSS).
  - Pre-equilibrate your dish of appropriate culture medium at desired protein concentration for final recovery of specimen.
- Remove the TS dish from 37°C incubator without CO_2 right before the warming procedure.
- While waiting for exposure time in TS, DS, and WS, cover the dish and move from the light source.
- For more gradual exposure of specimen to the solutions, particularly of oocytes, consider having substantial "carryover" of the previous solution when transitioning between drops until specimens are in final recovery medium.
- Following complete recovery 2–4 hours post-warming (full spindle reformation), oocytes must be fertilized by ICSI due to zona hardening during vitrification.

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