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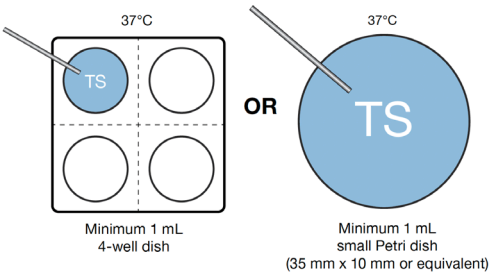
Blastocyst Fast Warming Protocol for Fast Warm - NX **(P/N 90196)**

The following protocol is for use of Fast Warm - NX (P/N 90196) with blastocyst stage embryos stored on vitrification devices that require direct exposure to Thawing Solution.

Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure.

01 **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 CO2 or on a heating stage at least 30 minutes prior to starting warming procedure.

Figure 1:



02 Identify the device sample(s) to be warmed and quickly transfer from LN2 storage to an LN2 filled holding reservoir in preparation for warming procedure.

03 Place LN2 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.

04 Prepare the device for warming by referring to corresponding device IFU and internal laboratory procedure(s).
Laboratory should consult their own procedures and protocols.

05 Remove TS dish from 37°C incubator without CO2 or heating stage and place it under focus on top of the microscope stage.

06 After specimen(s) is 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.

07 There are two options for warmed blastocyst(s):

For immediate transfer to patient:
Transfer blastocyst(s) to pre-equilibrated transfer medium.

For further culture:
Transfer blastocyst(s) to pre-equilibrated culture medium and incubate accordingly until desired developmental stage has been reached for transfer to patient.

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Tips for Blastocyst Warming

01 **Recovery medium:** Pre-equilibrate your dish of appropriate culture medium at desired protein concentration for final recovery of specimen.

02 Remove the TS dish from 37°C incubator without CO2 right before the warming procedure.

03 While waiting for exposure time in TS, cover the dish and move from the light source.

04 For a more gradual exposure of specimen to the solutions consider having substantial "carryover" of the previous solution when transitioning specimens into the final recovery medium.

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.

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