FUJIFILM
Value from Innovation

IrvineScientific

Simplified Warming Protocol for CryoTip

Oocytes and Embryos

ALL PROCEDURES 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 12mg/ml for final recovery of specimen(s).

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

1. Select CryoTip(s) to be warmed from LN_2 storage and quickly transfer to LN_2 filled holding reservoir in preparation for warming procedure.
2. Place LN_2 filled holding reservoir close to 37°C waterbath (minimum volume 500 mL) and microscope for subsequent rapid manipulation.
3. To set up warming dish, aseptically dispense (as shown in diagram):
   - One (1) 50 µL drop of TS
   - One (1) 50 µL drop of DS

   For oocytes, dispense a minimum of 100 µL of TS.
4. Quickly remove CryoTip from LN_2 and within 1 second fully immerse the device in the 37°C waterbath (>500 mL) and swirl gently device for 3 seconds. Swirling the device is critical to ensure the most rapid warming rate (+24,000°C/min).
5. Remove CryoTip from the waterbath and promptly remove metal cover sleeve from device by firmly grasping the lower end of the cover sleeve and pulling away from the CryoTip. Gently wipe away any water with a sterile dry tissue ensuring the tip of the device is dry.
6. Using sterile medical grade sharp scissors make Cut #1 below seal at wide end of CryoTip.
7. Withdraw the plunger of the syringe (with connector attached) to the half way position. Gently attach CryoTip to connector and syringe (or pipette).
8. Place fine tip end over the prepared warming dish and quickly make Cut #2 above the seal at the fine end.
9. While visualizing under the microscope, dispense contents of CryoTip as a small drop directly adjacent to TS drop. Once you visualize the specimen(s) touch the CryoTip contents drop to TS drop with end of CryoTip to mix. Set timer for 1 minute and leave undisturbed.
10. Transfer specimen(s) to DS for 4 minutes. Gently pipette specimens once to ensure complete rinse in DS.
11. During the 4-minute exposure in DS, aseptically dispense two (2) 50 µL drops of WS (WS1, WS2).
12. Transfer specimen(s) to WS1 then WS2 for 4 minutes each undisturbed.
13. 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.

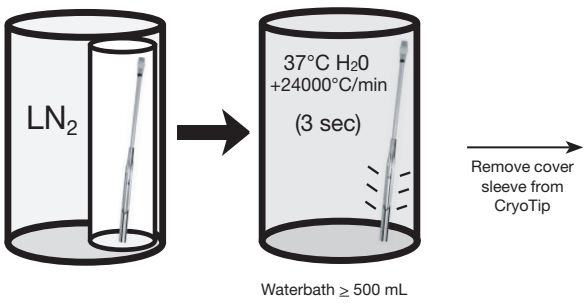
    There are two options for warmed EMBRYO(S):

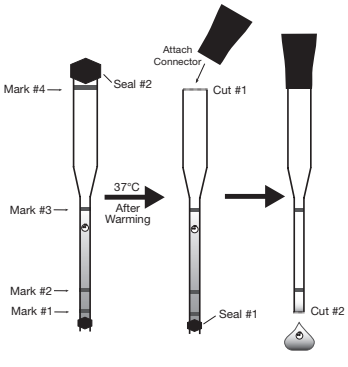
    a) For immediate transfer to patient: transfer EMBRYO(S) to pre-equilibrated "transfer" medium containing 20% (v/v) protein supplement or 12 mg/mL.

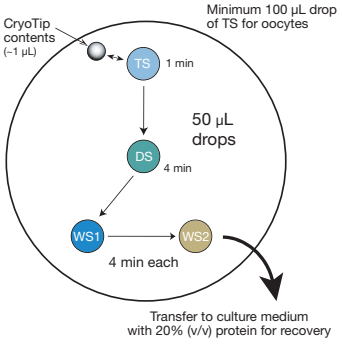
    b) For further culture: transfer EMBRYO(S) to pre-equilibrated culture medium containing 20% (v/v) protein supplement or 12 mg/mL for a 4 hour recovery period. After recovery period transfer EMBRYO(S) to culture medium with 10% (v/v) protein and incubate accordingly until desired developmental stage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

See reverse side for tips.

KEY			
TS	Thawing Solution	LN_2	Liquid Nitrogen
DS	Dilution Solution	↔	Merge drops
WS	Washing Solution	→	Transfer specimen to next drop







Simplified Warming Protocol for CryoTip Tips

- Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure.
- Pre-equilibrate a 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.
- CryoTips must remain submerged in LN_2 until ready to warm. When transferring CryoTips from LN_2 filled holding reservoir, or between LN_2 storage tanks, CryoTips should always be submerged in a LN_2 filled goblet to prevent uncontrolled/premature warming in air.
- Use sterile medical grade sharp scissors.
- Set up warming dish with drops of solutions (see step #3) prior to removing CryoTip from LN_2.
- Use a 37°C waterbath with a minimum volume of at least 500 mL.
- Ensure that the plunger of the syringe has been withdrawn half way prior to attaching the CryoTip to the connector.
- Rapid and controlled dispensing of contents from the CryoTip is essential, and requires a secure seal between the CryoTip, Connector and syringe (or pipette).
- Cut fine tip end over the dish in case of premature dispensing of contents. Carefully dispense contents as a drop (ideally hanging on edge of CryoTip before touching directly next to TS drop) to AVOID BUBBLES.
- Limit exposure to light while moving the specimens through the solutions.

Following complete recovery (2–4 hours post-warming), oocytes must be fertilized by ICSI due to zona hardening during vitrification.

www.irvinesci.com

FUJIFILM
Value from Innovation

IrvineScientific

FUJIFILM IRVINE SCIENTIFIC – CORPORATE
1830 E Warner Avenue, Santa Ana, CA 92705 USA
Phone: 1 (949) 261-7800
Toll Free: 1 (800) 437-5706
Fax: 1 (949) 261-6522
Support: tmrequest@irvinesci.com

FUJIFILM Irvine Scientific, the FUJIFILM Irvine Scientific logo, and CryoTip are registered trademarks of FUJIFILM Irvine Scientific, Inc. in various jurisdictions.

© 2019 FUJIFILM Irvine Scientific P/N 002048 Rev.00