

Semen and Sperm Freezing Protocol

Freezing Medium TYB and Arctic Sperm Cryopreservation Medium

Sperm freezing has become a routine process that depends greatly on the survivability and usability of the sperm after preservation. We've provided recommended protocols for Freezing Medium – TYB (Catalog # 90128) and yolk-free Arctic Sperm Cryopreservation Medium (Catalog # 90170) for your convenience*. Please refer to each product's product insert for storage information.

FREEZING MEDIUM TYB: RECOMMENDED PROTOCOL

Freezing Medium - TYB is a semen cryopreservation medium containing TEST Yolk Buffer (TYB), glycerol, and gentamicin.

1. Semen should be collected by masturbation following 3 days of abstinence.
2. Allow sample to liquefy at 37°C for 30 minutes. Measure the volume of the ejaculate.
3. A 5 mL vial of previously aliquoted Freezing Medium - TYB should be thawed and brought to room temperature or 37°C.

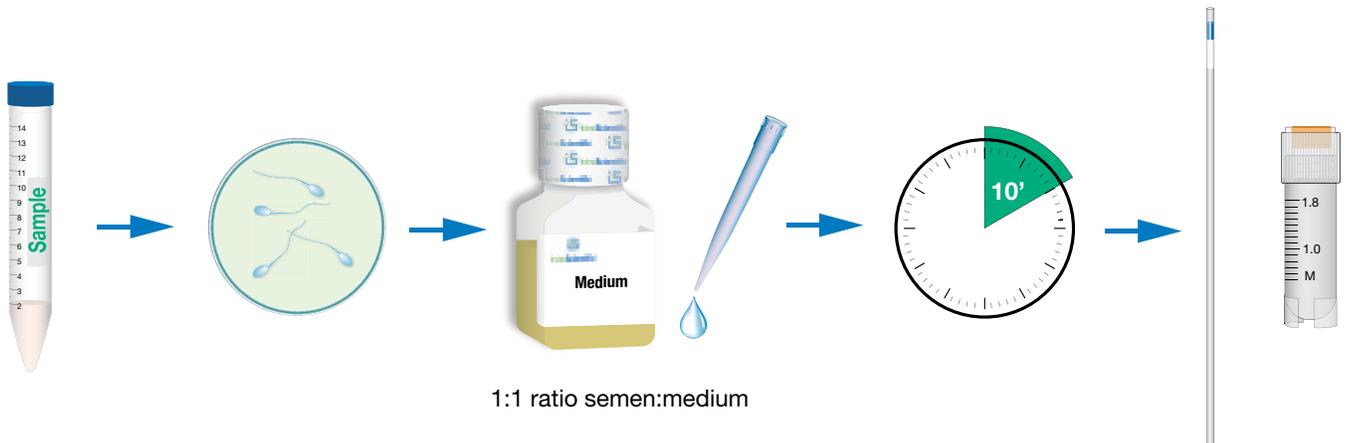
(Optional) After collection and liquefaction, the semen specimen may be cryopreserved as a raw specimen or it can be processed with a density gradient (ISolate - Catalog # 99264) and washed with Sperm Washing Medium (Catalog # 9983) prior to mixing with freezing medium. Please refer to the sperm separation protocols.

4. The liquefied sample is transferred to a sterile, 15 mL, conical centrifuge tube, the ejaculate volume is determined, and Freezing Medium-TYB added drop-wise, slowly, over a 30-second period until a 1:1 ratio of semen sample to medium is achieved. Mix it thoroughly after each drop is added so it equilibrates adequately while being careful not to create bubbles.

 Samples displaying high viscosity may require the additional step of repeated pipetting or passage through an 18 gauge needle to ensure thorough mixing.

5. Allow the mixture to equilibrate for approximately 10 minutes at room temperature.
6. Transfer/aspirate the final mixture into the patient-labeled storage vessel of your choice (cryostraws or cryovials) according to the manufacturer's filling protocol. To allow for expansion, do not overfill the container(s). Seal the device according to the manufacturer's recommended protocol and start the freezing process (see Sperm Storage Vessel Freezing Process).

 For cryostraws, put the cryostraw in a horizontal position to reach a homogeneous distribution of LN₂ vapor temperature along the cryostraw and to avoid unequal spermatozoid distribution due to potential spermatozoid sedimentation in cryostraw.



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ARCTIC SPERM CRYOPRESERVATION MEDIUM: RECOMMENDED PROTOCOL

Arctic Sperm Cryopreservation Medium is free of egg-yolk and antibiotics.

1. Semen should be collected by masturbation following 2–3 days of abstinence.
2. Allow sample to liquefy at room temperature or 37°C for 15–30 minutes. Measure the volume of the ejaculate.
3. One vial of Arctic Sperm Cryopreservation Medium is brought to room temperature or 37°C. If antibiotics are desired, they may be added at this step.
4. The liquefied semen sample is transferred to a sterile 15 mL conical centrifuge tube. The ejaculate volume is determined and an appropriate volume of Arctic Sperm Cryopreservation Medium is added drop-wise, slowly, until a 3:1 ratio of semen sample to medium is achieved. For example, for each 1 mL of semen, add 0.33 mL of medium.

 *To prevent osmotic shock, use a 1 cc sterile pipette to reduce the size of the drop, especially if semen volume is low (up to 1 mL).*

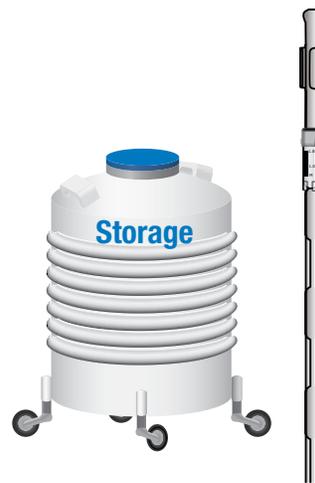
5. Transfer/aspirate the final mixture into the patient labeled storage vessel of your choice (cryostraws or cryovials) according to the manufacturer's filling protocol. To allow for expansion, do not overfill the container(s). Seal the device according to the manufacturer's recommended protocol and start the freezing process (see Sperm Storage Vessel Freezing Process).

 *For cryostraws, put the cryostraw in a horizontal position to reach a homogeneous distribution of LN₂ vapor temperature along the cryostraw and to avoid unequal spermatozoid distribution due to potential spermatozoid sedimentation in cryostraw.*

Sperm Storage Vessel Freezing Process

The freezing process from room temperature (20–25°C) to -80°C can be accomplished either by a programmable freezing system or by manually assisted vapor phase cooling

- For programmable freezing systems, use according to the manufacturer's instruction manual.
 - For the manually assisted vapor phase cooling method, mimic the freezing procedure below:
 - Load the cryostraws/cryovials to the cryocane, immerse into a beaker of water at ambient temperature, and place in the refrigerator (2–5°C) for 90 minutes.
-  *This step is skipped by some laboratories when it is not required by the validated procedures and protocols that have been specifically developed and optimized for their own medical programs.*
- Place cryocane over liquid nitrogen vapor for 30 minutes by either suspending them in the liquid nitrogen storage tank above the liquid level or placing them in the vapor phase in a small temporary liquid nitrogen dewar.
 - The final step should be to transfer the cryostraws/ cryovials quickly onto a labeled cane and then into the liquid nitrogen tank for storage at -196°C.



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* FUJIFILM Irvine Scientific has not validated these procedures and 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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