Semen and Sperm Freezing Protocol

Freezing Medium TYB and Sperm Maintenance 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 Sperm Maintenance Medium (Catalog # 99176) for your convenience. Both media are stored at -10°C. Do not expose medium to repeated freeze-thaw cycles. If smaller aliquots are desired, thaw the product, aliquot working volumes into sterile labeled containers, and freeze until needed.

**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 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. **A 5mL 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 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**

   **Note:** 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 manufacturers 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).**

   **TIP:** For cryostraws, put the cryostraw in a horizontal position to reach a homogeneous distribution of LN vapor temperature along the cryostraw and to avoid unequal spermatozoid distribution due to potential spermatozoid sedimentation in cryostraw.
Semen and Sperm Freezing Protocols

Freezing Medium TYB and Sperm Maintenance Medium

Sperm Maintenance Medium Recommended Protocol

Sperm Maintenance Medium (SMM) 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 a previously prepared aliquot of Sperm Maintenance Medium with Glycerol is thawed and 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 15mL conical centrifuge tube. The ejaculate volume is determined and an appropriate volume of thawed Sperm Maintenance 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.

   **TIP:** To prevent osmotic shock, use a 1cc 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).

   **TIP:** For cryostraws, put the cryostraw in a horizontal position to reach a homogeneous distribution of LN 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, the cryostraws/cryovials should be attached to the cryocane, and placed in the refrigerator (2-5°C) for one hour, then exposed to liquid nitrogen vapor for 30-60 minutes by either suspending them in the liquid nitrogen storage tank above the liquid level or by 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.

* 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.