

## THAWING PROTOCOL

**Principle:** The thawing protocol that provides optimal post thaw recovery of motile cells from a cryopreserved sperm sample is determined by the cryopreservation methods. The room temperature thawing method below has been tested to maximize post thaw recovery of motile sperm from samples cryopreserved by Cryobiology, Inc. All post-thaw QC sperm information is reported using this method.

**Thawing Temperature:** Room Temperature (approximately 23-24°C). Do not use water bath.

### Procedure:

1. Verify the identification and location of the vial(s) to be thawed.
2. Wearing protective goggles and gloves, find the appropriate cane. Lift the cane above the liquid nitrogen level only as far as needed to expose one vial. Remove the vial, re-submerge the cane.
3. Immediately place the vial on a countertop to begin thawing at **room temperature** (23-24°C).
4. After 5 minutes of thawing, loosen the cap on the vial to release any internal pressure created by the liquid nitrogen; retighten.
5. Thaw sample for at least 30 minutes.
6. If sperm motility appears sluggish after 30 minutes, allow to thaw for an additional 5 minutes and observe again.

### Technical Notes:

1. Samples must reach room temperature or warmer before handling to permit sperm cells to equilibrate.
2. Do not attempt wash procedures or other handling until thawing is complete and sperm cells exhibit forwardly progressive motility.
3. **Expiration Date:** There is no expiration date for this tissue if maintained at nitrogen vapor temperature or colder. Once thawed, the sample should be used within 8 hours.
4. Freezing Medium (cryopreservative) is sterile filtered and contains glycerol and egg yolk (not over 20% by volume).
  - Samples processed before September 1998 contain Penicillin-G:1000 units/ml and Streptomycin Sulfate:1000 µg/ml.
  - Samples processed after September 1998, contain Gentamicin Sulfate: 75 µg/ml.
  - NOTE: ICI samples have a final volume of 1.0 ml; IUI and ART samples have a final volume of 0.5 ml.
  - Patients with allergies to egg yolk or any of these antibiotics should consult their physician before using these samples.

**Sample Types:**

- **IUI** (Intrauterine insemination) Samples were developed for use in Intrauterine Insemination and do not contain seminal plasma.
  - i. **Expected Post Thaw Recovery:** Volume: 0.5 ml; approximately 20 million motile cells/ml (+/- 20%); minimum of 50% forward progressive motility.
- **ART** (Assisted Reproductive Technology) Samples were developed for use in In Vitro Fertilization and may require further processing. **ICSI** (Intracytoplasmic Sperm Injection) should be used with these samples; we do not recommend the use of ART samples for standard insemination in IVF.
  - i. **Expected Post Thaw Recovery:** Volume: 0.5; approximately 10 million motile cells/ml (+/- 20%); minimum of 30% forward progressive motility.
- **ICI** (Intracervical Insemination) Samples were developed for use in intracervical insemination using a cervical cap. They cannot be used for intrauterine or ART without additional processing since they contain seminal plasma. Processing will reduce the number of motile sperm cells available for insemination. ICI samples are not available on all donors as this sample freezing method was discontinued in September 1998.
  - i. **Expected Post Thaw Recovery:** Volume: 1.0 ml; approximately 30 million motile cells/ml (+/- 20%); minimum of 40% forward progressive motility.

**Note:** Results will vary based on counting chamber, counting technique and operator training and experience in analyzing sperm samples. These recoveries are for comparison purposes only.