The following protocols are for the thawing of semen or sperm processed using Sperm Maintenance Medium (Catalog # 99176) and Freezing Medium – TYB (Catalog # 90128). After thawing, please refer to specific instruction on how to handle the samples for intrauterine insemination (IUI) and in vitro fertilization (IVF).*

**Thawing protocol for raw semen**

1. Remove frozen specimen(s) from storage tank to thaw. Cryovials and cryostraws should warm for at least 15 minutes to room temperature.

2. While waiting for the specimen to thaw, label a 15 mL conical-bottom tube with at least 2 patient identifiers.

3. When the specimen has thawed, wipe the specimen container dry and open according to instructions for use from the manufacturer of the container.

4. Layer 1.5-2.0 mL of ISolate® lower layer and then an equal volume of ISolate upper layer into the tube using a sterile pipette, according to product insert instructions. Cap tube and place in warming block at 37°C while specimen thaws.

5. When the specimen has thawed, wipe the specimen container dry and open according to instructions for use from the manufacturer of the container.

6. Use a 1 cc pipette to layer the specimen from the cryovial onto the prepared two-layer ISolate gradient. For cryostraws, layer the specimen onto a single layer ISolate gradient to account for the smaller specimen volume.

**Note:** Add 2mL of Sperm Washing Medium (Catalog # 9983) or Multipurpose Handling Medium® – Complete (MHM*-C, Catalog # 90166) at room temperature to the sample slowly to avoid shocking/swelling the sperm before placing over the gradient.

7. Centrifuge for 20 minutes at 300 xg. Do not use the brake.

8. Remove the layers by inserting a clean 5 mL pipette tip just below the surface of the liquid.

**TIP:** Hold the tip in this position during aspiration. Aspirate the layers without disturbing the sperm pellet at the bottom of the tube until approximately 0.5 mL of lower layer remains. Even if a sperm pellet is not visible, this volume should contain sperm. If the sperm pellet occupies more than 0.5 mL at the bottom of the tube, aspirate as much liquid from above the pellet as possible, but leave the pellet intact.

9. Using a new sterile pipette, aspirate the pellet intact and put in a new sterile tube.

10. Using a new sterile pipette, add 2-3 mL of Sperm Washing Medium or MHM-C to the tube and resuspend the pellet. Centrifuge at 300 xg for 10 minutes. Do not use the brake. Remove the supernatant with a clean pipette.

11. Repeat step 10 for a second wash.

12. Using a sterile pipette tip, perform count and motility on specimen and record values.

13. After the second wash, discard the supernatant and resuspend the pellet in 0.25-0.5 mL of Sperm Washing or MHM-C if the sperm will be used for IUI. If the sperm will be used for IVF/ICSI resuspend in (CSCM, HTF, or P-1). Place the tube containing the washed sperm in a warming block, or water bath if it is to be used for IUI or into a CO₂ incubator if the specimen will be used for IVF/ICSI.
Sperm Thawing Protocols
Raw Semen and Processed Sperm

Thawing procedure for processed sperm

1. Remove frozen specimen(s) from storage tank to thaw. Cryovials and cryostraws should warm for at least 15 minutes to room temperature.

2. While waiting for the specimen to thaw, label a 15 mL conical-bottom tube with at least 2 patient identifiers.

3. When the specimen has thawed, wipe the specimen container dry and open according to instructions for use from the manufacturer of the container.

4. Transfer the entire contents of the cryovial or cryostraw to a 15mL conical-bottom tube pre-labeled with the patient’s name.

Note: If you are thawing from Sperm Maintenance Medium, you may skip to step 9.

5. Drop-wise, slowly add 5mL of Sperm Washing Medium or Multipurpose Handling Medium – Complete at room temperature.

6. Seal the tube and mix by repeated gentle inversion.

7. Centrifuge at 400 xg for 10 minutes. Do not use the brake.

8. Then remove the supernatant with a clean pipette, leaving about 500μL of medium and the sperm pellet. Discard supernatant and resuspend the pellet in 0.5-2mL of Sperm Washing Medium or MHM-C.

9. Using a sterile pipette tip perform count and motility on specimen and record values.

10. Place the tube containing the washed sperm in a warming block, or water bath if it is to be used for IUI or into a CO₂ incubator if the specimen will be used for IVF/ICSI.

After thawing, the sample can be processed for the desired technique.

IUI
For IUI, a second wash with 3mL of insemination medium (MHM-C or Sperm Washing Medium)* can be used. Centrifuge at 300-400 xg for 10 minutes. Do not exceed 400μL final volume for insemination.

IVF/ICSI
For IVF/ICSI, a gradient protocol can be used to isolate the motile fraction. For Irvine Scientific’s ISolate® density gradient medium protocol, refer to the sperm separation protocol.

For directions on using Irvine Scientific’s Sperm Washing Medium and MHM®-C, please refer to the respective product insert.

* 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.

For customer support contact us at tmrequest@irvinesci.com