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Sperm Thawing Protocols

Raw Semen and Processed Sperm

The following protocols are for the thawing of raw semen or processed sperm using Arctic Sperm Cryopreservation Medium (Catalog # 90170), or 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 stand for 5 minutes at room temperature and then placed into a waterbath at 37°C for 10 minutes.
2. While waiting for the specimen to thaw, label a 15 mL conical-bottom tube with at least 2 patient identifiers.
3. 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.
4. When the specimen has thawed, wipe the specimen container dry and open according to instructions for use from the manufacturer of the container.
5. 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.

   Add 2 mL 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.

6. Centrifuge for 15–20 minutes at 300 xg. Do not use the brake.

   The centrifugation time and speed may be adjusted accordingly (200 to 300 xg) to the individual specimen quality to optimize the procedure.

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

   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.

8. Using a new sterile pipette, aspirate the pellet intact and put in a new sterile tube.
9. 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 8–10 minutes. Do not use the brake. Remove the supernatant with a clean pipette.
10. Repeat step 9 for a second wash.
11. Using a sterile pipette tip, perform count and motility on specimen and record values.

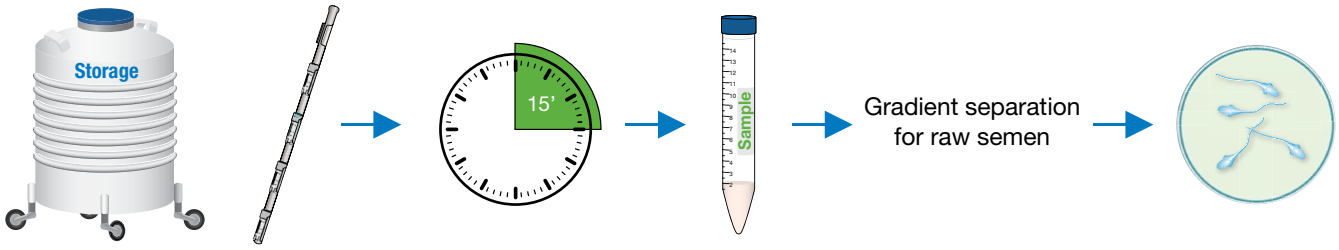
The Sample Can Be Processed For The Desired Technique

IUI

Discard the supernatant and resuspend the pellet in no more than 0.5 mL of Sperm Washing Medium or MHM-C. Place in a warming block or water bath.

IVF

Discard the supernatant and resuspend sperm to desired dilution in fertilization medium (CSCM, HTF, or P-1). Place the tube containing the washed sperm into a CO_2 incubator.



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 stand for 5 minutes at room temperature and then placed into a waterbath at 37°C for 10 minutes.
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 15 mL conical-bottom tube pre-labeled with the patient's name.
5. Slowly add 5 mL of Sperm Washing Medium or Multipurpose Handling Medium-Complete at room temperature.
6. Mix gently with a pipette.
7. Centrifuge at 300 xg for 10 minutes. Do not use the brake.

   The centrifugation time and speed may be adjusted accordingly (200 to 300 xg) to the individual specimen quality to optimize the procedure.

8. Remove the supernatant with a clean pipette down to the sperm pellet.
9. Resuspend the sperm in Sperm Washing Medium or Multipurpose Handling Medium-Complete, perform count and motility on specimen using a sterile pipette tip and record values.

The Sample Can Be Processed For The Desired Technique

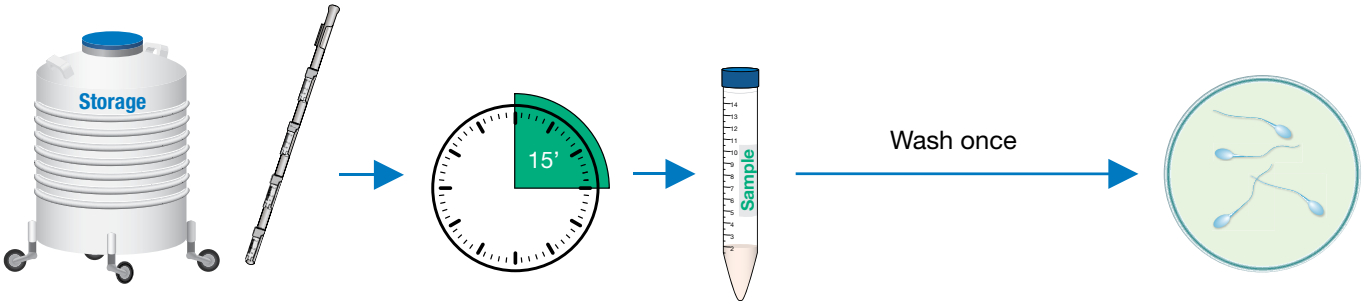
IUI

For IUI, a second wash with 3 mL of insemination medium (MHM-C or Sperm Washing Medium) can be used. Centrifuge at 300–400 xg for 10 minutes. Discard the supernatant and resuspend the pellet in no more than 0.5 mL of Sperm Washing Medium or MHM-C. Place in a warming block or water bath.

IVF

If a gradient has not been used before specimen freezing, a gradient protocol can be used post thaw to isolate the motile fraction. For use of FUJIFILM Irvine Scientific's ISolate density gradient medium refer to the Sperm Separation Protocol (P/N 001771). Discard the supernatant and resuspend sperm to desired dilution in fertilization medium (CSCM, HTF, or P-1). Place the tube containing the washed sperm into a CO_2 incubator.

For directions on using FUJIFILM Irvine Scientific's Sperm Washing Medium and MHM-C, please refer to the respective product inserts.



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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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