



INTERNATIONAL ANIMAL SEMEN BANK, INC.
dba INTERNATIONAL CANINE SEMEN BANK and ICSB
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Thawing ICSB Frozen Canine Semen

Please read the following instructions as soon as you receive this shipment!

ALWAYS CONFIRM THE CONTENTS OF THE TANK ARE FOR THE APPROPRIATE BREEDING! IF THERE IS ANY CONCERN, CALL 503-663-7031 IMMEDIATELY!

PROPER TANK HANDLING AND SEMEN STORAGE

The frozen semen you are receiving is being shipped in a dry shipper tank, which usually does not contain any measurable liquid nitrogen. The tank has been charged with liquid nitrogen and should hold liquid nitrogen temperature (-196 °C) for several days. This shipper has one canister, in which both the thaw media and frozen semen have been placed. Usually, one thaw media will accompany each vial of frozen semen. The thaw media has been placed on an aluminum cane(s) with a blue cane tab. The frozen semen has been placed on an aluminum cane(s) and the top of its cane labeled “semen”, the name of the dog, or the name of the dog’s owner.

When the shipment arrives, the tank should be checked for vapor by removing the neck cap/plug by pulling it straight up out of the tank. When this is done, vapor should be seen in the neck of the tank. This indicates that the contents are still frozen. **Replace the cap immediately after checking for vapor.** If the insemination is to be done in a day or two, and provided the tank has not been damaged in shipment, the semen should remain frozen until used. One or two liters of liquid nitrogen can be added to the tank, as well. Keep the tank upright and capped in a temperature-moderated room away from heat sources.

When transferring the semen from the shipper tank to the storage tank, do so very quickly, as vials of semen may contain some liquid nitrogen that could cause it to burst if left out of the liquid nitrogen. **Always use appropriate protective gear when utilizing the tank and its contents.**

HOW TO MAKE A SLIDE WARMER AND WATER BATH FOR THE THAWING OF SEMEN

If a water bath and slide warmer are unavailable, the following instructions are provided to create a temporary water bath and slide warmer:

A simple water bath can be made from a large pan or bowl and thermometer. Fill the bowl about $\frac{2}{3}$ full with warm water at 100°F. When it is time to use the water bath for thawing the pellets, check temperature and adjust with hot or cold water to bring the temperature to 100°F.

A slide warmer can be made using a block of metal, plastic, or glass with a smooth, flat surface. Place the block in the water bath so that the surface is about $\frac{1}{2}$ -inch above the water. Place the microscope slide on this block to warm, then put a drop of the thawed semen on the slide and cover with a slip.

See Back for Thaw Instructions



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THAW INSTRUCTIONS

PREPARING THE THAW MEDIA

- 1. Wear appropriate protective gear, including cryogenic-protective eyewear and gloves.**
2. Remove one vial of thaw media and let thaw at room temperature about 30 minutes prior to the procedure.
3. Wipe the vial dry and add thaw media to the provided Whirl-Pak thaw bag.
 - ⇒ Use as much thaw media that the volume, once in combination with the semen, will reach the optimum insemination volume for the insemination procedure that is being performed.
4. Place the bag into a 98°-100°F water bath for at least two minutes prior to retrieving the frozen semen.

RETRIEVING THE SEMEN

The frozen semen is in a screw-cap vial. If there is any liquid nitrogen in the vial, you must keep the vial in the tank until it is opened to avoid the vial bursting.

- 1. Wear appropriate protective gear, including cryogenic-protective eyewear and gloves.**
2. Lift the semen cane to the top of the tank neck.
3. Observe the vial to be sure no liquid is in the vial: if you shake the cane, the pellets of the semen will be loose and rattle in the vial.
 - ⇒ If there appears to be liquid in the vial, keep the vial in the neck of the tank:
 - i. Grasp the vial of semen while still attached to the cane and slightly unscrew the cap with a gloved hand.
 - ii. Unclip the vial from the cane.
 - iii. Hold the vial at an angle until all the liquid has evaporated.
4. **Rapidly** completely unscrew the vial and pour the pellets into the prepared thaw bag so that the pellets drop **directly** into the warmed thaw media.
 - ⇒ Tap the bottom of the vial to be sure all the pellets have dropped into the media.
5. Quickly reach into the water bath and massage the thaw bag until all of the pellets have dissolved.
 - ⇒ **DO NOT LET WATER COME INTO CONTACT WITH THE SEMEN!**
THIS WILL DAMAGE THE SEMEN!
6. Do not leave the thawed semen in the water bath for longer than 5 minutes. If the AI is going to take longer than 5 minutes, remove the thaw bag and let it sit at room temperature.
7. The semen should be evaluated prior to AI to ascertain the quality. This should be performed with a body temperature microscope slide:
 - i. Gently mix the semen.
 - ii. Remove one drop and place it on a warm slide.
 - iii. Cover with a slip and let sit for about 10 seconds.
 - iv. Observe the semen at 100X.
8. Slowly draw the semen into the syringe, being careful not to draw air up through the semen.
9. Tip the syringe up and draw ½ - 2 cc of air in, which will be used to discharge all of the semen from the insemination apparatus at the time of AI.