

International Canine Semen Bank- Grass Valley, Inc
Po Box 741
Cedar Ridge, CA 95924
(530) 273-9123 Phone
(530) 273-9128 Fax

THAWING ICSB FROZEN CANINE SEMEN DRY SHIPPER- SCREW CAP VIAL SYSTEM

READ IMMEDIATELY UPON ARRIVAL!!!

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 (-196degrees C) for several days. This shipper has one canister, in which the frozen semen has been placed. *For your thaw media, use sterile saline, approximately 1.5-2cc.*

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. At this point, the shipping tank should either be filled with liquid nitrogen or the semen and thaw media removed and placed in a semen storage tank.

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.

When preparing to thaw the semen for insemination, draw up 1 ½- 2 cc sterile saline and pour into the accompanying Whirl-Pak thaw bag. Place the bag into a 98-100 degree Fahrenheit water bath for at least 2 minutes prior to retrieving the frozen semen.

The frozen semen is in a screw-cap vial. If there is any liquid nitrogen in the ampoule, you must keep the vial in liquid nitrogen until it is opened. The vial may burst if it is taken out of the liquid nitrogen prior to opening.

When retrieving the frozen semen, you will need to wear protective eye ware and loose fitting leather gloves. **Occasionally, the vials may fall off the canes in shipment. So make sure they are on the cane, and not in the bottom of the canister.** Lift the cane marked “Semen” to the top of the tank neck. Observe the vial to be sure no liquid is in the vial. If you shake the cane, the pellets of 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. With a pair of sponge forceps, grasp the vial of semen while attached to the cane and unscrew the cap with a gloved hand. Unclip the vial from the cane. If you see liquid in the vial, or hear a sizzling sound, there is still liquid nitrogen in the vial. Hold the opened vial in the air until all the liquid has left, then rapidly invert the vial into the thaw bag so the pelleted semen drops into the warmed media. Quickly reach into the water bath and massage the thaw bag until all the pellets have dissolved. **ALWAYS INSURE NO WATER GETS IN THE BAG OF SEMEN OR MEDIA!!!**

DO NOT LEAVE THE THAWED SEMEN IN THE WATER BATH FOR LONGER THAN 1 MINUTE!!! Remove the thaw bag with thawed semen, tip the thaw bag so that the semen flows into one corner of the bag and then slowly draw the semen into the syringe, being careful not to draw air up through the semen. Put the syringe up into the opposite corner and 'sweep' the inside of the bag down to the corner again and draw up any remaining semen. **The semen should be left at room temperature, not in the water bath until the procedure is performed. This should be no longer than 10 minutes from the semen thaw.**

The semen should be evaluated prior to the AI or surgical implant to ascertain the quality. **This should be performed with a body temperature microscope slide!! Not a cold slide!!** Gently mix the semen, remove one drop and place it on a warm slide, then coverslip. Observe the semen at 100X and estimate the percent motility, progressive motility (based on a 0-5 scale, 5 being rapid forward progression), and percent bent sperm. (The percent of moving sperm cells, **on a warmed slide** that exhibit a circling movement or backward movement.)

HOW TO MAKE A SLIDE WARMER-WATER BATH FOR THE ABOVE PROCEDURES:

WATER BATH: A simple water bath can be made from a large pan or bowl and thermometer. Fill the bowl about 2/3 full with warm water at 100 degrees and check with the thermometer. When it is time to use the water bath for thawing pellets, check temperature again and adjust with hot water or cold water to bring the temp to 100 degrees.

SLIDE WARMER: A slide warmer can be made by obtaining a piece of metal, plastic, or glass with a smooth surface which can be placed in the water bath. (Small laboratory racks work also) The smooth surface should stick up above the final water level about 1/2 inch. A microscope slide or slides, can be placed on this block and should reach the same, or very closely the same, temperature as the water.

Use metal or plastic pan, preferably flat bottomed. Make sure temperature of water before using to thaw the frozen semen is 98-100 degrees.

Use a metal, plastic or glass block as a slide warmer. Be sure one side is smooth and flat to place the microscope slides on. Set the block in the water bath. It will warm to the water temperature.

Artificial Insemination: Usually, we have been advised ahead of time as to which procedure is being performed, and we adjust the thaw media volume accordingly. In this event, you may have to adjust the volume of media.

If using our standard non-surgical artificial insemination procedures, use 4-6cc sterile saline.

If you elect to perform the surgical implant (recommended), the frozen semen pellets should be thawed in about 1.5ml of thaw media(sterile saline) for small dogs and 2cc of thaw media (sterile saline) for larger dogs. Too much volume can actually flush the eggs right out! Half of the volume should be injected into each horn, making sure no resistance is felt when depressing the syringe plunger. Also be sure to inject the sample SLOWLY, again to insure that the eggs are not flushed out.

TANK RETURN: The shipping tank is to be returned prepaid and empty. UPS or Fed Ex are the preferred carriers. In most cases, we will have enclosed a completed airbill for your convenience. You will need to date the airbill and call the carrier for pickup.

PLEASE NOTE: For domestic shipments, a rental fee is charged for the first 7 days. If the shipper tank is not returned within this period, a daily rental fee of \$10.00 is charged until the tank is returned. Please return the tank as soon as possible to avoid additional charges.

If you have further questions, please contact us:

ICSB-Grass Valley, Inc

Bridgett Higginbotham, RVT,

(530) 273-9123 Phone (530)273-9128 Fax cell: 530-913-4957

Email: info@icsbgrassvalley.com website: www.icsbgrassvalley.com