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**INTERNATIONAL CANINE SEMEN BANK - INDIANAPOLIS
(ICSB – INP)**

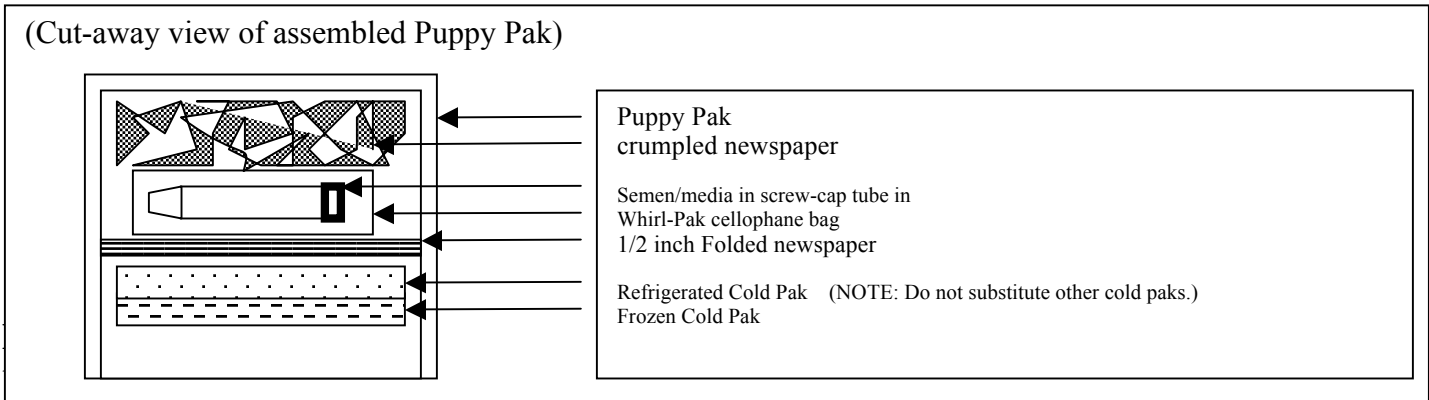
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**INSTRUCTIONS FOR USING THE ICSB CANINE
FRESH SEMEN SHIPPING KIT ("PUPPY-PAK")**

When you receive the kit, place ONE cold pak and the media in your freezer and ONE cold pak in the refrigerator. The media is packaged in a frozen state. It may have thawed in transit. This will not harm the media if it is immediately refrozen after it arrives. If you are ordering more than one kit, all media are usually in one kit. (NOTE: Rarely, the media tube(s) will leak in transit. The cellophane bag will catch the media if this does occur. Open the bag, remove and open the media tube, then pour the contents of the bag back into the tube, recap, and freeze.)

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When you wish to use the shipping kit for shipping fresh semen:

1. Place the frozen pak in the bottom of the kit and the refrigerated cold pak on top of the frozen pak.
2. Place about 1/2 inch of paper packing over the two cold paks.
3. Thaw the media and warm to between 70 and 98 degrees Fahrenheit. This can be facilitated by placing the tube in a warm water bath between the above mentioned temperatures. Mix the tube of media well so it is evenly mixed. Gently add the collected semen from the stud dog. Evaluate prior to shipping. NOTE: Remove approximately 0.3 ml of the mixed semen, place this in a test tube in the refrigerator and check the motility the next morning after warming to room or body temperature for 5 minutes. Prepare a slide/ coverslip and let warm to body temperature before observing motility microscopically.
4. Place the screw cap on the tube and enclose it in the cellophane bag it came in. Fold the wire end of the bag several times over itself, then fold the wire ends across the bag. This provides a seal that will prevent leakage out of the bag in the event the tube might leak. Place in kit on top of the 1/2 inch of newspaper.
5. Place crumpled newspaper on top of the semen tube to hold the semen and cold paks in place.
6. Enclose the necessary paperwork, instructions, tape securely and ship.



1. Use clean apparatus that is not contaminated with soap or other residues.
2. Collect the semen at room temperature (no lower than 65 degrees Fahrenheit).

Once the dog starts to ejaculate, the normal progression of events is for the ejaculate to first appear as a clear liquid, then turn to a milky white, then back to a clear fluid. If you are using the ICSB-OR collection kit, this will easily be seen in the collection tube.

3. Once the milky portion of the ejaculate is obtained and the clear third portion begins to appear, cease collecting from the dog.
4. Add the semen to the shipping media (which should be between 65 and 98 degrees Fahrenheit), place the screw cap back on the tube securely and package the semen as per the instructions.
5. Ship either United Parcel Service (preferred), or via Federal Express Priority One. We use UPS primarily because there seems to be better temperature control in their cargo hold.

### **ARTIFICIAL INSEMINATION OF THE SHIPPED CANINE SEMEN**

When the shipped semen arrives, it should be evaluated for motility prior to insemination. In some cases, the shipped semen can be divided for at least two inseminations, 24 hours apart, if desired. This is determined by microscopic evaluation for quantity and percent motility after the semen arrives for the insemination.

1. Remove the semen from the small bag, mix gently and place the tube of semen-media in a body temperature water bath (No warmer than 98 degrees Fahrenheit) for 5 minutes, then
2. mix the semen gently by inverting the tube slowly 2-3 times.
3. Place a small drop of the semen on a body temperature (97 to 98 degree F.) microscope slide. Place a coverslip on the specimen, let warm at body temperature for 30 seconds, then quickly transfer to a microscope and observe motility at 100X power. If the motility appears low, set the semen-media in a 90 degree F water bath and take a single drop to evaluate at 5 minute intervals, letting the slide warm to body temperature prior to each observation.
4. Mix the specimen gently again and draw the semen into your insemination equipment, being careful not to draw air up through the semen as you load.
5. Deposit the semen vaginally at the opening of the cervix. Elevate the bitch's hindquarters for 5 to 10 minutes after insemination to allow the semen to pool at the cervix.

If you are using the ICSB artificial insemination kit, follow the instructions for depositing the semen at the cervical os. If you have any questions regarding the procedures, or wish to order any of the supplies, please feel free to write or call.