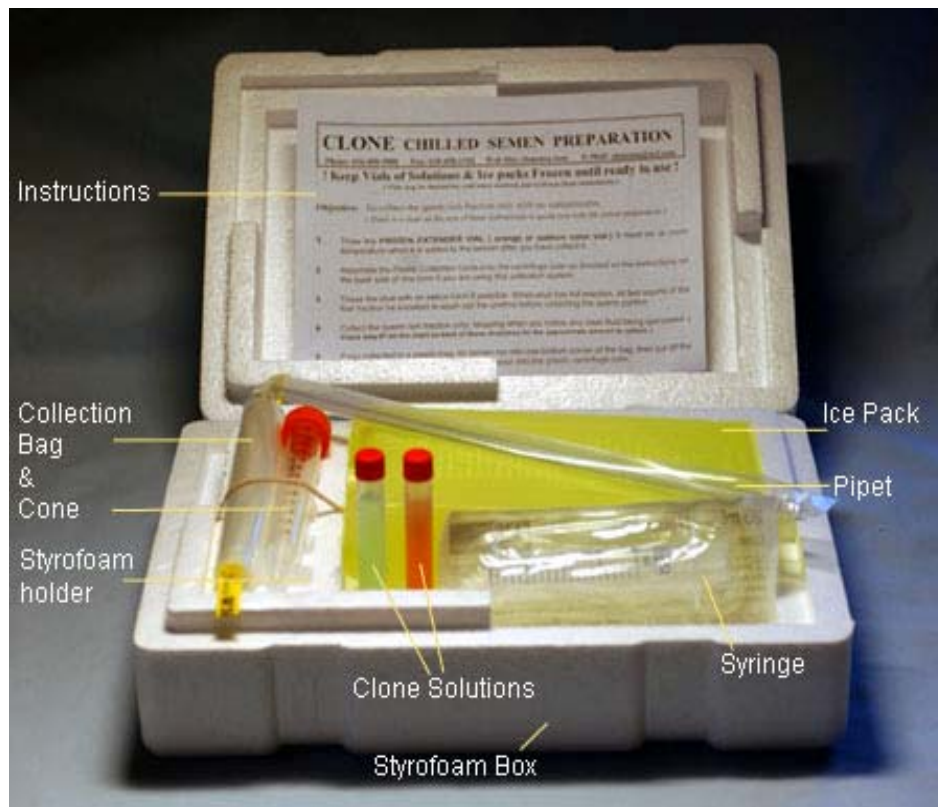


CLONE CHILLED SEMEN PREPARATION

! Keep Vials of Solutions & Ice packs Frozen until ready to use !

Vials may be thawed, but cold, when received. Just put the vials, as well as the ice pack, immediately in the freezer and keep there until they will be used.



Objective: **To collect the sperm rich fraction only with no contaminants.**

1. Thaw the FROZEN EXTENDER VIAL

(orange or salmon color vial) to room temperature for about **1 hour** before you collect the semen. Have all collection equipment prepared, if using a plastic bag have it opened and ready.

2. Assemble the Plastic Collection Cone onto the centrifuge tube as directed on the instructions on the back of the form.

3. Use an estrus bitch to tease the stud if possible. When stud has full erection, let two squirts of the first fraction be expelled to wash out the urethra before collecting the sperm portion.

4. Collect the sperm rich fraction only, stopping when you notice any clear fluid being

ejaculated. The semen is collected, trying to collect only the sperm rich fraction of the collection. If this cannot be accomplished without collecting a great deal of prostate fluid, the collection should be **centrifuged** (see step # 8.) to separate the sperm cells from the other fluids that were collected. At this point all other fluids are drawn off, leaving only the sperm cells and a small amount of seminal fluid behind. This is done to avoid the problems with some studs that have blood, contaminates or prostate fluid that may have toxic effects on the semen. If we assume that all dogs could have problems associated with their semen, by removing all the prostate and/or supportive fluids from the semen collection we can control and insure that all semen processed will be as uniform as possible, thus averting problems that could be encountered by the novice collector of the semen.

5. If you **collected in a plastic bag**, let semen run into one bottom corner of the bag, then cut off the other bottom corner of the bag.

6. Pour the semen into the plastic **centrifuge tube**.



7. **The semen evaluation should be done at this time.**

8. If step # 4 can't be accomplished without collecting a large amount of prostate fluid, you will have to **centrifuge the semen** at urine speed (700 g; very slow) for 3 to 4 min. There should be no more fluid then 1 ml for small dogs & 1.5 ml for medium & large dogs to add the Orange coloured Extender.

- Draw off the supernate with a syringe very slowly, leaving about .25 to .50 ml of prostate behind.

- The remaining pellet of sperm cells are **re-suspend** into the remaining prostate fluid by gently rocking the centrifuge tube until sperm cells are totally back into solution, then **evaluated** and then processed with the semen enhancer/preservative solution. In this way

any **prostate fluid problems are corrected** with correct supportive solutions and poor quality semen is thus improved.



9. Add the orange or salmon colored extender to the semen at this time, very slowly. The result is a semen collection improved and preserved for shipment.



10. Attach the centrifuge tube to its styrofoam holder with a rubber band, you could use 4 wraps of paper towels in lieu of the styrofoam holder.

Set the frozen ice pack next to the styrofoam semen holder, **keeping the tube of semen from touching the ice pack** by facing it away from ice pack.

11. Close and seal the shipping box, making sure that the Semen Activator vial (clear solution), Insemination Instructions, Syringe & Insemination Pipet are included in the box for shipment to the inseminator of the bitch.

12. Tape the box closed and ship by way of Next Day Air or faster. Do not use the US Postal service as it might not arrive in time. The semen will cool on it's way and be ready to be reactivated and inseminated when it arrives at it's destination.

! IMPORTANT !

- 1. Do not use any other equipment** in contact with semen, other than that included in the kit.
- 2. Avoid any temperature shocks** to the semen when adding enhancer or activator. If it is at a different temperature than the semen, the semen could get damaged or killed.
- 3. Avoid any fluctuations of temperature**, up then down, up then down.

CLONE CHILLED SEMEN **INSEMINATION**

1. **When semen arrives, keep refrigerated**, (not in freezer) until ready to use.

At this point, before warming it up properly, the **semen is in an almost motionless state!**

2. **One half to one hour before the insemination bring the semen and the activator** (the clear or cloudy solution vial) out of the refrigerator and let them both slowly come up to room temperature.

3. **Prepare a water bath** of approximately 1 gallon of water, in a container such as a Styrofoam box or bucket. Have the water or bath pre-warmed to 85 to 90 degrees F.

4. **Add the activator solution** slowly to the semen (which is in the centrifuge tube). Both should be at the same temperature at this time.

5. Put the cap back on tightly, then **gently rock the semen tube** back and forth a few times very slowly until all the solutions are mixed.



At the semen portion is submerged, in the warm, 85 to 90 degree **water bath for 8 to 10 minutes** to activate the semen to motility and activity.

After **the semen** to check the motility and forward speed of

7. **Assemble** the breeding tube or rod to the syringe and draw the semen up the breeding tube into the syringe in one column with no air bubbles.

8. Inseminate the bitch in the elevated position, and keep her rear elevated for 8 to 10 minutes after the insemination is performed.

! IMPORTANT !

🔥 **Never warm semen above the temperature of 90 degrees Fahrenheit (37 C).**

🔥 **Inseminate the bitch as soon as you take the semen out of the water bath.**

🔥 **Inseminate at room temperature and not in any cold area after the semen has been prepared and warmed.**