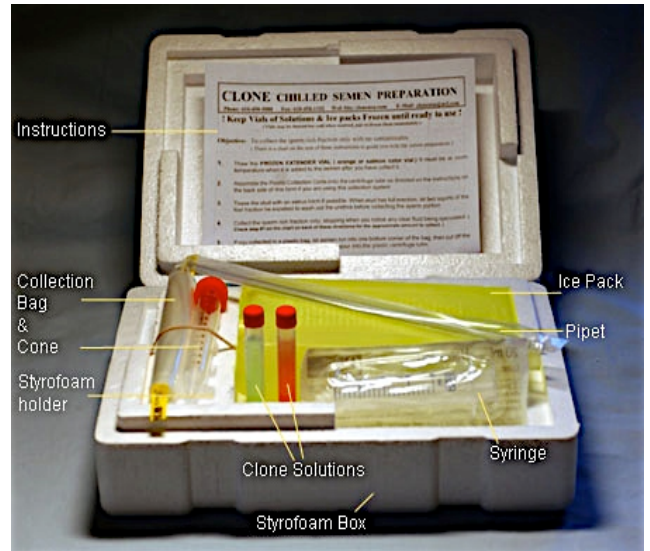


## CHILLED SEMEN PREPARATION

When your CLONE Chilled Semen Kit arrives keep the vials of solutions & ice packs frozen until ready to use. The vials may be thawed, but still cold, when received. Just put the vials, as well as the ice pack, immediately in the freezer and keep them there until they are to be used.

1. For best results you should aim to collect only the sperm rich fraction of the stud dog's ejaculate without any contaminant.
2. Thaw the Frozen Extender (orange or salmon coloured vial) to room temperature for about 1 hour before you collect the semen. Have all collection equipment prepared. Have the special plastic collection bag opened and ready with the top rolled back the top to make a soft collar. Do not touch the inside.
3. Use an in-season bitch to tease the stud dog if possible. When dog has full erection, let two squirts of the first fraction be expelled to wash out the urethra before collecting the sperm rich portion into the plastic collection bag. As far as possible minimize contact between the penis and the bag to reduce contamination.
4. Collect the white or creamy coloured sperm-rich fraction only. Stop when you notice any clear fluid being ejaculated. If this cannot be accomplished without collecting a great deal of clear prostate fluid that follows the sperm-rich fraction, the collection should be centrifuged (see step # 6.) to separate the sperm cells from the other fluids collected. At this point all other fluids are drawn off, leaving only the sperm cells and a small amount of fluid behind. This is done to avoid the problems with some studs that have blood, contaminants or prostate fluid that may have toxic effects on the semen. If we assume that all dogs could have problems associated with their semen then, by removing almost all the prostate and/or supportive fluids from the semen collection, we can control and ensure that all semen processed will be as uniform as possible, thus averting problems that could be encountered by the novice semen collector.
5. Allow the semen run into one bottom corner of the bag, then cut off the other bottom corner and through this carefully pour the semen into the plastic orange topped centrifuge tube. If semen evaluation can be performed it should be should be done at this time.
6. If step 4 can't be accomplished without collecting a large amount of prostate fluid, you will have to centrifuge the semen to remove the excess fluid. This is done at the speed urine is centrifuged to collect deposits (700 g / very slow) for 3 to 4 minutes. There should be no more fluid then 1 ml for small dogs & 1.5 ml for medium & large dogs to which you will add the Orange Coloured Semen Extender.



7. Draw off the supernatant with a syringe very slowly, without disturbing the 2plug” of sperm at the bottom of the tube. Leave about ¼ to ½ ml of the fluid behind and then replace the cap and resuspend the sperm in this fluid by gently rocking the centrifuge tube until fully resuspended. The semen is then evaluated and processed with the Semen Extender solution. In this way any problems caused by prostate fluid are corrected with the supportive solutions and poor quality semen may be improved.



Add the orange or salmon colored Semen Extender to the semen very slowly. The result should be a semen collection improved and preserved for shipment

Wrap the centrifuge tube with 4 wraps of paper towels and secure with a rubber band.

8. Set the frozen ice pack(s) back in the Styrofoam box and add the semen sample in its paper towel wrap. Keep the tube of semen from touching the ice pack

9. Close and seal the shipping box, making sure that the Semen Activator vial (clear or cloudy / bluish solution) included, still frozen in the box not in contact with the semen. Include the insemination syringe, Insemination Catheter and Insemination Instructions with the shipment. Insemination Instructions, Insemination Syringe & Insemination Pipette are included in or with the box for shipment to the inseminator.
10. Tape the box closed and ship by way of Next Day Air or faster. Do not use the regular 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.

**Note: For shipment by air counter to counter delivery with collection of the package form the airport is, in our experience, quicker and more reliable than a door to door service.**

### **IMPORTANT**

- Do not use any other equipment in contact with semen, other than that included in the kit.
- Avoid any fluctuations of temperature. Ideally collect the semen in a room or environment at a stable temperature around 20 – 24 Centigrade
- Avoid subjecting the semen to any temperature shocks especially when adding the Semen Extender (and the Semen Activator). If it is at a different temperature to the semen, the semen will be damaged and may be killed.