



CANINE-EXT™ EXTENDER FOR FRESH CHILLED SEMEN

Suggested Procedures:

1. Preparation of extender:
 - Add powder to provided double-distilled, sterile water. Mix well, until powder is completely dissolved.
 - Add 20ml of fresh egg yolk and 3ml glycerol. Mix all very well. (see the separate description for procedure to prepare egg yolk)
2. Collect sperm rich fraction.
3. Extend in Canine-EXT™ mixture at room temperature.
 - If sperm concentration of raw ejaculate is:
300-500 x 10⁶ml = Dilute 1 part semen + 3 parts extender.
500-800 x 10⁶ml = Dilute 1 part semen + 4 parts extender.
800 x 10⁶ml = Dilute 1 part semen + 5 parts extender.
4. Cool for 60-75 minutes to 4-5°C. This can best be done by:
 - Tightly sealing a tube of extended semen.
 - Fill a beaker with room temperature water. (volume of water should be approximately 10x that of the extended semen in the tube)
 - Place tube of extended semen in beaker with water.
 - Place beaker in 4-5°C refrigerator and allow to cool slowly. *Caution: Do not allow any of the beaker water to contaminate the extended semen !*



CANINE-EXT™ EXTENDER cont.

6. Add an equal volume of pre-cooled (4-5°C) Part B Extender until the final concentration = 200×10^6 sperm/ml.
7. Fill and seal 1/2cc straws.
8. Freeze straws. Note: There are varying ways and procedures to freeze semen (ie: freezing over vapor vs freezing direct). It is recommended that you do several test runs to determine what is the best combination of variables for your circumstances.

Thawing Semen for Evaluation

1. Thaw straws in 36-37°C water bath for 30 seconds.
2. Pat dry straws.
3. Empty straw contents into 1ml of Part C Extender at 36-37°C.
4. Allow to equilibrate for 5 minutes at 36-37°C, evaluate quality of semen.