

PRODUCT # 106022

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CANINE-EXT™ EXTENDER FOR SEMEN CRYOPRESERVATION

Suggested Procedures:

1. Preparation of extender:

- Part A - Initial Extender: Add powder to provided double-distilled, sterile water. Mix well, until powder is completely dissolved. Then add 20ml of fresh egg yolk and 3ml glycerol. Mix all very well. (see the separate description for procedure to prepare egg yolk)
- Part B - Freezing Extender: Add powder to provided double-distilled, sterile water. Mix well, until powder is completely dissolved. Then add 20ml of fresh egg yolk, 7ml of glycerol and 1ml of Equex™ and mix well. [See the separate instructions for the recommended procedure to prepare egg yolk. Note: The Equex™ is a thick solution. Equex™ can be prewarmed at 35-37° C to liquify; then pipette or use a TB syringe to measure desired volume.]
- When you are ready to thaw, mix Part C, Thawing Extender: Add powder to provided double-distilled, sterile water. Mix well, until powder is completely dissolved.

2. Collect sperm rich fraction.

3. Centrifuge @ 700xg for 6 minutes at room temperature.

4. Dilute pellet at room temperature in Part A Extender to 400×10^6 sperm/ml.

5. 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 !*



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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.