



Culture Medium for Canine Semen

# Chill 10 Protocol



Ref. 13574/0210

Store at 4°C

## Extender Preparation:

- Warm Canipro ApX<sup>2</sup> Chill 10 to room temperature.
- Add 20% fresh egg-yolk to volume needed of Chill 10. The volume needed of extender will be 3 to 5 times the volume of second fraction collected. Gently mix. See Preparation of Egg Yolk on side two for directions.

## Semen Extension:

- Use only the **sperm-rich fraction** of the ejaculate (2nd fraction) for insemination. Do **not** include the first fraction of the ejaculate (clear prostatic fraction before sperm rich fraction) or the third fraction (prostatic fraction post-sperm rich fraction) of the ejaculate in the sample because the quality of the semen will decrease.
- After collection evaluate semen quality (Concentration, Motility, Morphology).
- Dilute one part of semen in three to five parts of extender. (ie: 1ml in 3-5ml of Canipro Chill 10 based on initial semen concentration).

Canine Semen Concentration (10 <sup>6</sup> ) Per ML	Ratio of Semen to Extender
250 – 750	1 to 3
750 – 1.25	1 to 4
Above 1.25	1 to 5

- Semen can be preserved for up to 10 days at 4°C (39°F) with preservation of a minimum of 70% initial motility. Avoid changes in temperature during preservation by placing the tube with the extended semen in small water-bath.

- At time of insemination warm semen to room temperature and perform a motility evaluation (See insemination instructions below).
- **For purpose of shipping chilled semen** - Store diluted semen at 4°C (39°F) for at least 2 hours before packaging and shipping.
- Perform a motility evaluation before shipping and retain a control aliquot for future evaluation.
- If needed to reach ideal insemination volume (See insemination instructions below) add some extender in extra 13ml tube in **Canine Transport Box**.

**Note for washing of semen:** If the ejaculate collected is contaminated with prostate fluid, wash the semen with Canipro ApX<sup>2</sup> Chill 10 by diluting 1 part of semen in 3 to 5 parts of extender (i.e. 1 ml of semen in 3 to 5 ml of Canipro ApX<sup>2</sup> Chill 10) based on concentration. Centrifuge at 700g for 10-15 minutes. After centrifugation, discard the supernatant and slowly re-suspend the sperm pellet in 3 to 5 parts of Canipro ApX<sup>2</sup> Chill 10 (i.e. 1 ml of sperm pellet in 3 to 5 ml of extender) based on concentration.

## Insemination:

Vaginal, transcervical (TCI) or surgical insemination can be performed based on the semen quality evaluation. Use semen either chilled or warmed in a water bath to 37°C (99°F). Inseminate slowly, allowing time for absorption.

- **To evaluate motility**, aliquot a small sample and warm in a water bath to 37°C (99°F), then pipette onto microscope slide for evaluation.

*Continued on next side*



## Insemination Continued:

- **Vaginal insemination** with a MAVIC™ catheter: use the extended semen to ideally reach the following total volumes:

2-5 ml for dogs <10 Kg (< 22lbs)

5-10 ml for dogs between 10 - 20 Kg (22lbs - 44lbs)

Up to 20 ml for dogs > 20 Kg (> 44lbs)

**Note:** These volumes simulate the volumes of the second and third fractions produced during natural mating and may vary by breed.

At time of insemination first inject the extended semen, then flush with the extra volume of extender mimicking what happens naturally during the tie.

- **TCl or surgical insemination:** use 1-3 ml of extended semen depending on the size of the bitch.

## Preparation & Addition of Egg Yolk for CaniPRO ApX<sup>2</sup> Extender

- Obtain fresh eggs.
- Wash, rinse, dry and store in a refrigerator until use.
- Place a metal egg separator over a 400 ml beaker.
- Crack the egg and separate the yolk using the above separator and beaker.
- When the yolk and white have separated sufficiently, place the yolk with membrane intact on a folded square of paper towel.
- Roll the yolk very gently to remove any excess white. The egg yolk can be aspirated from the membrane using a 5 ml syringe and a 16-18 gauge needle. Alternatively the yolk membrane can be lanced and the yolk drained into a beaker or measuring cylinder. It is very important that the yolk membrane not be used in the extender, as it may be harmful.
- Carefully add the exact amount of egg yolk required by the extender using a calibrated instrument (syringe, pipette, etc).

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