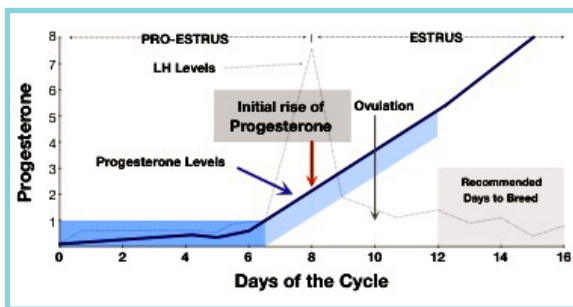


INITIAL RISE OF PROGESTERONE DETERMINES BREEDING DATES



The visible signs of heat and breeding time (such as vulval swelling, end of vaginal discharge, or flagging) are only an approximate indication of the time of ovulation. These signs can vary by more than a week.

Progesterone levels begin to rise at the same time as the LH surge. Ovulation occurs two days after the initial rise in progesterone. **Predicting ovulation is done accurately by detecting the initial rise in progesterone.**

With the TARGET Canine Ovulation Timing kit, it is possible to detect the initial rise in progesterone. As seen in the figure, the progesterone level before ovulation is low (between 0 and 1.0 ng/ml). This corresponds to a bright blue (C1) test result. When the initial rise in progesterone occurs, the test result will be a light blue (C2).

OVULATION TIMING GUIDELINES

In order to determine when to breed, It is most important to determine the day when the **initial rise** in progesterone occurs. This is the day when the test changes from a C1 to a C2 color. Record all color results on a calendar.

1. Test a baseline sample at the first sign of vulval swelling and bleeding. (Helpful hint. To check for first bleeding press a white paper towel firmly to the vulva and examine for blood.)

At baseline level which is usually between 0-1 ng/ml progesterone, the test will show a bright blue color result similar to C1 on the color chart. **Record the color result on a calendar.**

2. **At 5-7 days** after observing the first day of vaginal bleeding and vulval swelling, **start testing every other day.** Record the first day when the results change from a bright blue C1 color to a lighter blue C2 color on the calendar. This is the initial rise. Ovulation will occur 1-3 days later. Plan to inseminate in 3-6 days after the **C1 to C2 color change.**

3. After observing the color change from C1 to C2, continue testing every day until the test results show faint blue C3 color. A C3 result means ovulation has occurred and the egg is beginning to ripen and the most fertile time to breed is 2-4 days later. This result in Step 3 should indicate the same breeding date as the result in step 2.

4. If a white C4 result is obtained, mate or inseminate immediately.

C-SECTION GUIDELINES

During pregnancy progesterone is present at high levels to maintain fetus viability. Progesterone is highest at 30 – 60 ng/mL around day 25. It declines very slowly until about 3 days prepartum. When progesterone falls to less than 2 ng/mL (C2) about 24 hours before birth, then a lung surfactant necessary for breathing is released. Whelping is imminent, and a C-Section can be scheduled within 24 hours.

Target is a useful tool for timing a C-Section when it is used in conjunction with all other available information such as breeding date and other physical signs indicating readiness for delivery.

- Drop in rectal temperature to below 100 F (normal Temp 101 – 102.5 F)
- Restlessness with panting; uneasiness
- Seeking a quiet place to build a nest
- Mucus plug being discharged

Approximately 58-60 days after the first breeding (average gestation is 63 days), progesterone tests should be run.

When progesterone drops to light blue (C2) after a previous test was close to white (C4 or faint C3) and other physical signs indicate a readiness for delivery, a C-section delivery should be scheduled within 24 hours.

SAMPLE PREPARATION (plasma or serum) & HELPFUL HINTS

Collect the blood sample into a purple EDTA or heparin coated tube or a red top tube. Do NOT use a serum separator tube.

Spin the blood down with a centrifuge. Or allow the blood to clot at room temperature for 1/2 - 1 hr or until a clear serum collects in top of tube.

Label the sample with the name, sample day and cycle day.

Store the Kit in the Refrigerator.

To do a low control on the kit reagents, do steps 2-7, the result should be C1 since no progesterone is present.

Do not exchange test cups or reagents between different kits.

Progesterone values measured by Target are "Free" (Active) Progesterone in sera.

See targetvet.com for info on how free progesterone values relate to RIA (Free + Bound) progesterone values.

Intended for veterinary use only. Not for human use.
The manufacturer warrants the kit for its intended use.
BioMetallics' liability is limited to the value of the kit.



PROTOCOL

TEST PREPARATION

Take a test cup and kit solutions out of the refrigerator 10 minutes prior to using.

Use hand to warm cup and solutions to room temperature.

1 Add **2 drops sample*** to the center of the cup.
WAIT 2 MINUTES. Use a new pipette for each sample.



*For best results, collect sample in a Purple or Red Top Tube without any serum separator gel and spin down or wait for serum to separate.

2 Add **2 drops Sample Wash** with RED Label. Wait for liquid to drain into cup. REPEAT this step 1 time.



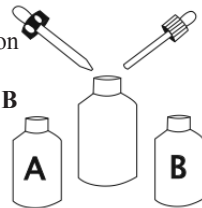
3 Add **1 drop enzyme** from the RED CAP BOTTLE to the center of the cup and WAIT ONE MINUTE - timing this step is important.



4 Fill cup reservoir with WASH solution from the WHITE label bottle.



5 Prepare fresh substrate solution by adding one dropper (filled to the mark) EACH of A and B into empty MIX bottle.



Shake MIX bottle well.

6 Add 2 drops FRESHLY prepared substrate from the MIX bottle to the center of the cup.*



*Freshly made substrate solution is good for 30 min. Discard any unused solution after the test is run and save mixing bottle for future tests.

7 Record results in 9 MINUTES.
Bright Blue (C1)
Light Blue (C2)
Faint Blue (C3)
or White (C4)



Rev. 11/11

Interpretation of the Results

The recommendations below are only a guideline and all the available information concerning the dog's cycle should also be considered.

The test color result corresponds to the free (active) progesterone level present in sera.

Use the color chart below for evaluating and recording the result. All values refer to "free" progesterone.

Color	Progesterone ng/ml	Recommendation
Bright Blue C1	0 - 1	Retest every two days until a light blue (C2) is obtained.
Light Blue C2	1 - 2.5	Retest every day until a faint blue (C3) is obtained. Plan to inseminate in 3 to 6 days.
Faint Blue C3	2.5 - 8	Repeat test daily. Breed or inseminate in approximately 1-3 days.
White C4	>8	Mate or inseminate immediately.

- C1 Baseline progesterone level
- C2 Initial Rise in progesterone has occurred. Fertile breeding period is 3-6 days later.
- C3 Ovulation has occurred (1-3 days after C2). Eggs begin to ripen.
- C4 Progesterone > 8 ng/ml (2-3 days after C3). Eggs are ripe and mature



CANINE OVULATION TIMING KIT

Rapid Canine Progesterone Kit

For Use In Veterinary Clinics

- Accurate timing is important for breeding success
- Improper timing is the main reason for unsuccessful breedings
- This kit will allow the user to determine the optimum breeding time

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