

USING TARGET TO DETERMINE THE MARE'S CYCLE

Progesterone, a natural hormone that circulates in the mare's blood, is produced by the corpus luteum (CL) and changes during a normal estrous cycle. **Progesterone levels in the blood accurately reflect the different stages of the estrous cycle.**

• No Cycling Occurs

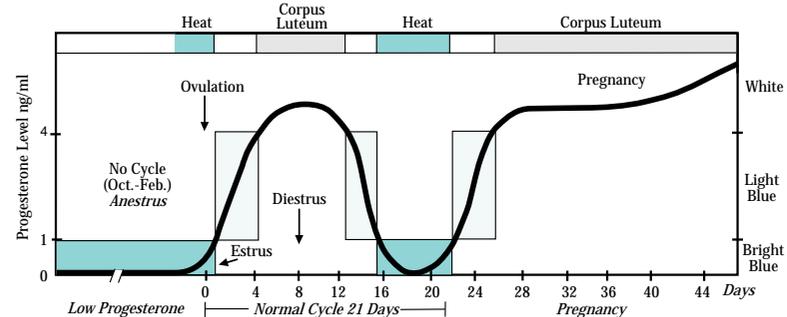
- **Winter Anestrus:** No ovulation occurs during this time and therefore there is no progesterone. The test result is BRIGHT blue.
- **Transition Period:** Developing follicles are detected by palpation. If ovulation has not occurred for the season, test result is BRIGHT blue.

• Cycling Begins After The First Ovulation:

- **Estrus:** The cycle begins. The normal cycle is 21-22 days. Each heat (estrus) lasts 5-7 days. Progesterone levels are low during estrus (see figure). The test result is BRIGHT blue.
- **After Ovulation (Day 0):** The CL forms on the ovary and begins producing progesterone and the Progesterone levels rise. The test result is LIGHT blue.
- **Diestrus:** The progesterone concentration is very high during the 14-15 days of the diestrus period. The test result is WHITE.

- In the unbred mare, the CL regresses around day 16. The progesterone levels fall and the cycle is repeated (see figure).
- In the pregnant mare, progesterone levels remain elevated beyond 17 days post breeding (see figure).

PROGESTERONE LEVELS IN THE MARE



WHEN TO USE TARGET EQUINE PROGESTERONE TEST

- For determining where the mare is in her cycle. In this way you can reduce or eliminate the need for teasing the mare. A progesterone test is the most economical way to determine where the mare is in her reproductive cycle.
- For determining whether there is a functional CL and thus, whether to use prostaglandin.
- About 72 hours after a prostaglandin injection, in order to determine if the treatment was effective or if a second treatment is needed.
- For monitoring the progesterone level during pregnancy.
- For early indication of pregnancy.
- For monitoring progesterone for embryo transfer.

USING TARGET WITH PROSTAGLANDIN

- Prostaglandin is used when the mare has a functional or persistent corpus luteum (progesterone level is high) in order to induce heat (low level of progesterone).
- In order for prostaglandin to be most effective, it is important to know where the mare is in her cycle at the time of injection in order to predict the result.
- If a bright blue result is obtained on a first test, test again in 4 days to find out if your mare is approaching heat or is still in anestrus.
- For an unbred mare, a white result indicates that the mare is in diestrus or may have a persistent corpus luteum. Prostaglandin may be used at the time of a white result to bring her into heat. About 72 hours after prostaglandin administration, a progesterone test will confirm heat (bright blue result). If the result is not bright blue, the CL has not fully regressed and could recover. Another treatment of prostaglandin is advisable in this case.
- For a mare that has been bred, a white result 17-23 days after breeding can indicate pregnancy. Prostaglandin should NOT be used at this time, since it lowers progesterone and will cause an abortion of the pregnancy.

USING TARGET WITH REGU-MATE

TARGET is designed to recognize only the natural progesterone hormone.

REGU-MATE is a synthetic hormone and will not be detected by TARGET. When using TARGET to monitor pregnancy while REGU-MATE is being given, a white result indicates the progesterone-deficient mare is producing enough progesterone to be taken off therapy.

USING TARGET TO MONITOR PREGNANCY

Low levels of progesterone at any stage of pregnancy are a major cause of early embryo loss or late-term abortion. To determine if progesterone levels are adequate for pregnancy, test a sample 12-14 days after ovulation. Retest at various intervals during pregnancy to insure adequate progesterone levels are being maintained.

- A white result indicates progesterone levels are adequate for pregnancy maintenance. The minimum safe level to maintain pregnancy is 4 ng/ml progesterone white.
- A bright blue (C1) or light blue (C2) result indicates a progesterone-deficient mare.

PREGNANCY DETERMINATION

TARGET can be used for pregnancy detection. A white result obtained 21-23 days after breeding indicates pregnancy. Note: TARGET indicates the presence of progesterone not specifically pregnancy. A positive indication of pregnancy should be confirmed by palpation or ultrasound.

USING TARGET FOR EMBRYO TRANSFER

TARGET can be especially useful in embryo transfer programs where following the estrous cycles closely is essential in synchronizing the donor with a recipient mare.

TEST PREPARATION

(SERUM, PLASMA, OR WHOLE BLOOD SAMPLE)

For a serum / plasma sample, collect blood into a labeled Red (serum) or a Purple (plasma) Top Tube. For serum, allow the tube to stand for 20-30 minutes at room temperature or spin the sample down in a centrifuge. Pour the clear liquid into a clean glass tube.

For a whole blood sample, collect at least 6 drops of blood into a red or purple top tube. Run the test within 5 minutes after taking the blood sample. Invert the tube several times to mix before running the sample. After 5 minutes the blood sample may begin to clot which may clog the test cup and cause invalid results.

OTHER IMPORTANT TIPS:

- Store this kit in the refrigerator when not in use. Do not freeze.
- Always reseal the plastic bag after removing a test cup.
- Timing during Step 3 (Time of the enzyme) IS IMPORTANT: one (1) minute.
- Do not exchange test cups or reagents between different kits.
- The color results should be a full circle. A half moon or crescent shaped color result should be recorded as the darkest part of the color.

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.

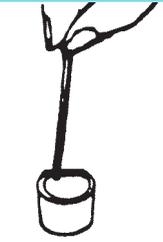
BioMetallics
Princeton, NJ USA

PROTOCOL

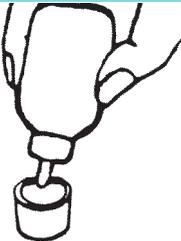
TEST PREPARATION

Take the kit out of the refrigerator at least 2 hours before using it in order to allow the kit to come to room temperature. *(It is possible to leave the kit at room temperature overnight, if desired.)*

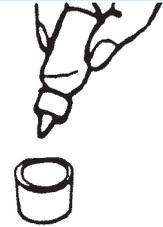
1 Shake sample. **Add 4 drops of serum (or fresh blood) sample to the center of the cup. WAIT 2 MINUTES.** Use a new pipette for each sample.



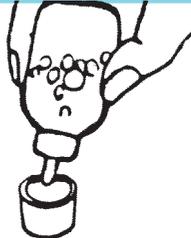
2 **Add 4 drops SAMPLE WASH (White Cap Bottle with RED LABEL). Wait for liquid to drain into cup. REPEAT** this step 2 times or until the cup is white.



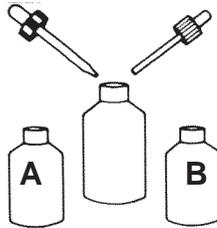
3 **Add 3 drops enzyme** from the RED CAP BOTTLE to the center of the cup and **WAIT ONE MINUTE** - timing this step is important.



4 Fill cup with WASH solution (WHITE CAP BOTTLE with WHITE LABEL) to the top of the inner line. Wait for liquid to drain completely into cup.

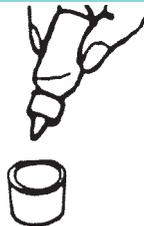


5 Prepare fresh substrate solution in empty BLUE CAP MIXING BOTTLE using one dropper (filled to the mark) EACH of Substrate A and Substrate B. Shake the freshly prepared substrate in the mixing bottle well.

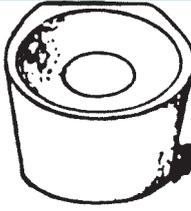


6 Add 4 drops FRESHLY prepared substrate (BLUE CAP) to the center of the cup.*

*After 30 minutes, throw out any unused solution in the blue mixing bottle. Save blue mixing bottle for future tests.



7 Record color results in 7 MINUTES: Bright Blue (C1), Light Blue (C2), Faint Blue (C3) or White (C4)



Rev. 03/01

INTERPRETING THE RESULTS

Color And Progesterone Level	Interpretation
Bright Blue C1 0 - 1 ng/ml	<p>No progesterone is present and there is no functional CL.</p> <ul style="list-style-type: none"> If the mare has not started her normal breeding cycle, a bright blue result indicates she is still in winter anestrus, or she may be entering the transitional phase, if developing follicles are present upon palpation. If the mare has started normal spring cycling, bright blue indicates she is in heat or estrus. The duration of estrus is 5 to 7 days with ovulation occurring 24 to 48 hours before the end of estrus. Since the average lifespan of semen in the female reproductive tract is 48 hours, mares are usually inseminated every 2 days until ovulation is detected by the presence of a light blue test result. <p>Progesterone is beginning to rise or fall (See Figure).</p>
Light Blue C2, C3 1 - 4 ng/ml	<ul style="list-style-type: none"> If the first test result is light blue, then test again in 2 days to determine if the mare is entering or leaving heat. If a previous test was bright blue, then a light blue result indicates progesterone is rising. The mare has ovulated 1 to 2 days before. If a previous test was white, then progesterone is dropping. The mare is approaching her estrus period and should be tested again in 2 days. <p>• white result indicates a high progesterone level and a functional CL is present.</p> <p>This means the mare has ovulated and is in diestrus or in a non-fer- tile period.</p>
White C4 > 4 ng/ml	<p>If the mare fails to exhibit signs of estrus during the breeding sea- son, a continued white result may indicate:</p> <ul style="list-style-type: none"> A persistent CL and prostaglandin treatment may be necessary <u>Q_R</u> Pregnancy if this white result is obtained 21-23 days after breeding.



EQUINE BREEDING SYSTEM

FOR THE DETERMINATION OF THE MARE'S CYCLE

Rapid Equine Progesterone Kit

For Use In Veterinary Clinics

- The mare's reproductive cycle is subject to great variability. Measuring the progesterone concentration permits the most accurate determination of where the mare is in her cycle and allows plan- ning the most effective reproductive program.
- Knowing the progesterone allows:
 - diagnosis of a functional or persistent corpus luteum;
 - the most effective use of prostaglandin and/or progestin treatment;
 - monitoring progesterone during the course of pregnancy



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