

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 lowest during estrus (see figure). *The test result is BRIGHT blue.*

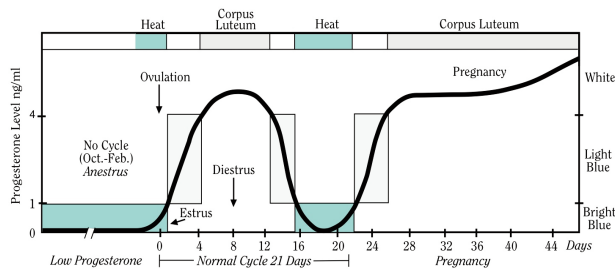
- **After Ovulation (Day 0):** The CL forms on the ovary and begins producing progesterone and the Progesterone levels rise. *The test result is LIGHT blue.*

- **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 results indicates progesterone levels are adequate for pregnancy maintenance. The minimum safe level to maintain pregnancy is 5 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.

SAMPLE PREPARATION (serum or plasma) & HELPFUL HINTS

Collect the blood sample into a red top tube or an EDTA or heparin coated purple 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 **4 drops sample*** to the center of the cup.
WAIT 2 MINUTES. Use a new pipette for each sample.



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.



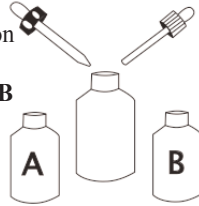
4

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 7 MINUTES.
Bright Blue (C1)
Light Blue (C2)
Faint Blue (C3)
or White (C4)



Rev. 5/10

INTERPRETING THE RESULTS

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

Color And Progesterone Level	Interpretation All values refer to "free" progesterone
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, <i>mares are usually inseminated every 2 days until ovulation is detected by the presence of a light blue test result.</i>
Light Blue C2 1 - 2 ng/ml	<p>Progesterone is beginning to rise or fall (See Figure).</p> <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.
Faint Blue C3 2.1 - 5 ng/ml	<ul style="list-style-type: none"> If a previous test was bright blue, then a light blue result indicates progesterone is rising. <i>The mare has ovulated 1 to 2 days before.</i> If a previous test was white, then progesterone is dropping. <i>The mare is approaching her estrus period and should be tested again in 2 days.</i>
White C4 > 5 ng/ml	<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-fertile period.</p> <p>If the mare fails to exhibit signs of estrus during the breeding season, a continued white result may indicate:</p> <ul style="list-style-type: none"> A persistent CL and prostaglandin treatment may be necessary OR Pregnancy if this white result is obtained 21-23 days after breeding.

 **TARGET**
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 planning 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 prostin treatment;

- monitoring progesterone during the course of pregnancy

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