

Early pregnancy detection is key to reducing feed and labour costs in modern farming.

The Bovine Pregnancy Rapid Test Kit provides a fast, reliable 15-minute solution by detecting pregnancy-associated glycoproteins (PAGs) in blood samples, enabling early and informed herd management decisions.

## 1. Application

For rapid bovine pregnancy testing in whole blood, serum, or plasma from approximately 28 days after mating or AI.

Not recommended within 70 days post-calving due to PAG persistence.

## 2. Kit Components

- Rapid Test Card, in foil pouch,                      Pipette.
- Sample buffer,
- Needle and EDTA blood tube,

## 3. Sample preparation

### Whole blood:

Collect blood into EDTA tubes and mix gently by inverting the tube approximately 10 times immediately after collection.

Store at 2–8 °C for up to 24 hours. Do not freeze whole blood samples.

### Serum:

Collect blood into tubes without anticoagulant and allow it to clot. Process the sample by centrifugation (e.g. 5000 rpm for 5 minutes).

Use the clear upper layer as serum. Store at 2–8 °C for up to 24 hours.

### Plasma:

Collect blood into EDTA tubes and mix gently by inverting immediately after collection. Process the sample by centrifugation (e.g. 5000 rpm for 5 minutes).

Use the clear upper layer as plasma. Store at 2–8 °C for up to 24 hours.

## 4. Operations

1) Read instructions before use. Use freshly collected samples and allow both test kit and samples to reach room temperature (20–30 °C) prior to testing.

2) Take test card needed from the kit package, and make proper marks. Please use the test strips within 1h. Return the rest into cold storage.

3) **Serum/Plasma sample:** Take **3 drop (90-120ul)** of **serum/plasma** sample into the sample well on the test card;

**Whole blood sample:** Take **1 drop (40ul)** of **whole blood** sample into the sample well on the test card, then add **2 drops (60ul)** of **sample buffer** with the buffer vial in the kit.

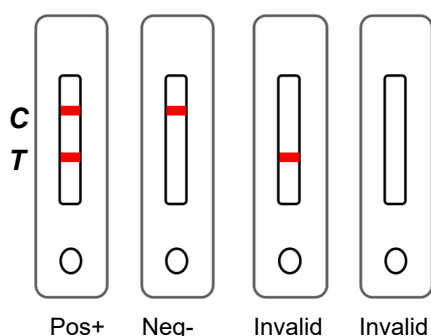
4) Wait for 15min and then determine the result according to **Part 5**. Results observed after 30min is invalid.



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## 5. Result determination

There are 2 lines on the test card, **Control Line**, **Test Line**. Test results will depend on the color of these lines.



**Positive (+):** Line C and line T are visible and red.

**Negative (-):** Line C is red, and Line T is not visible.

**Invalid (x):** Line C has no color. In this case, please read the instructions and repeat assay with new test card.

Incomplete control line or incomplete test line is still VALID.

Note: **positive result needs to be confirmed by testing of sample from the cow on the next day.**

In field test of more than 500 cows, this kit demonstrated 98.3% specificity and 97.5% sensitivity. The total accuracy is over 97%. However, since this is not a confirmatory test, positive results shall be confirmed by other methods.

### **IMPORTANT CAUTION FOR RESULT DETERMINATION**

- (1) For all samples testing on day 28 post AI, positive results will have a pale and weak test line, in either cases, if you can see a weak or very weak test line, the result is positive.
- (2) The test line will be more visible on day 35-40 or later post AI after the PAG level increases in blood.
- (3) The test line will be more visible on test cards using serum/plasma as samples due to the direct sample operation without dilution by buffer
- (4) The test line will less visible on test cards using whole blood as samples due to the dilution with sample buffer.
- (5) Test results with incomplete test line or incomplete control are still valid.
- (6) We suggest work with the vet to establish protocols using this test kit on your farm.





## 6. Storage

2-30°C in cool dark place, do not freeze. The kit is valid for 18 months.  
Lot number and expired date are printed on the package.

## 7. Notice and precautions for a successful experiment.

Please perform the assay following the instruction. Use the test card within 1h after opening the foil pouch. Wear gloves to perform the testing. This test card is used for only once; please do not use it repeatedly. Proper reproduction programs shall be made with the vet to better utilize the test kit.



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## 8. Limitation of the methodology

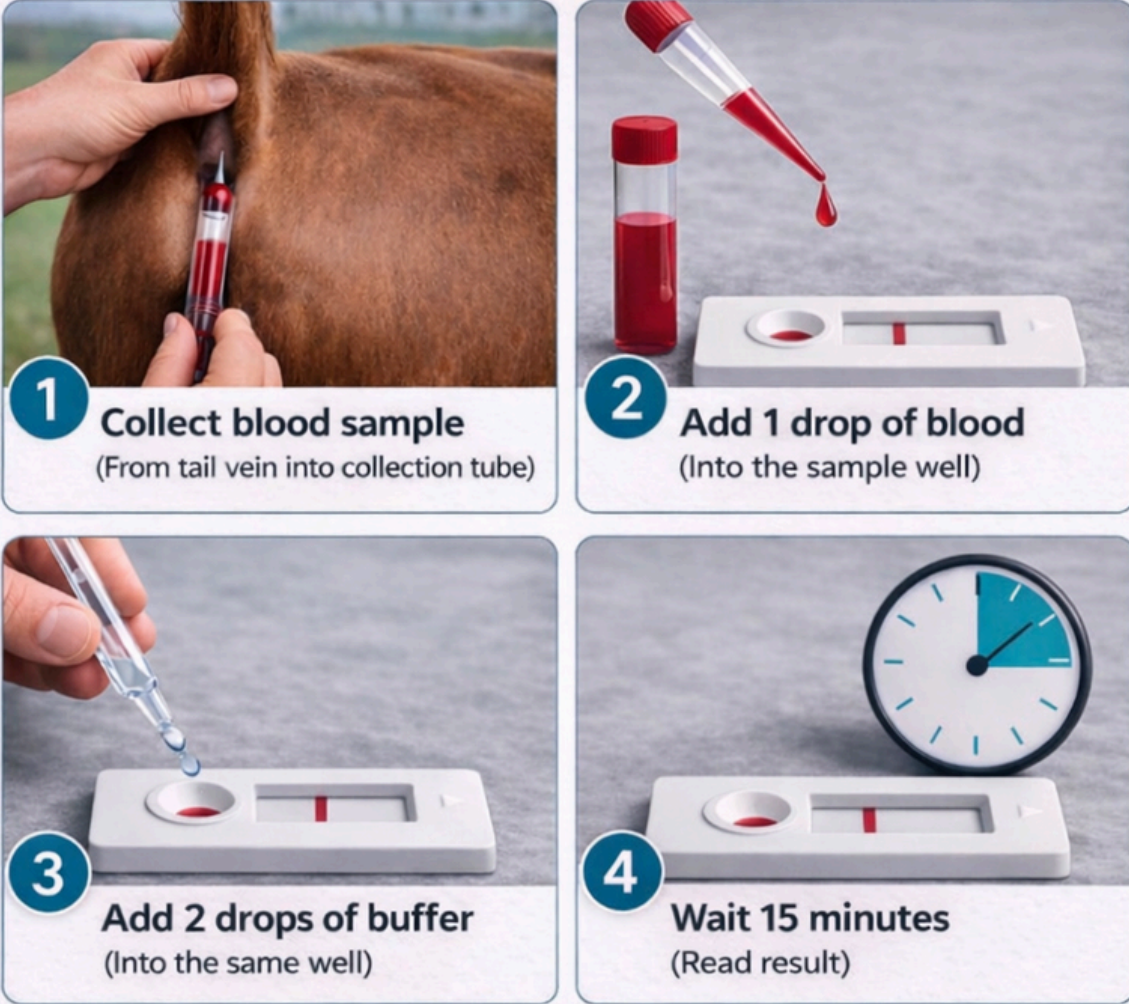
- 1) The kit is based on the detection of PAG at certain levels for the fast testing of pregnancy in cows. Thus, the test result may rely on the PAG levels in cows. For cows with 60 days post parturition, this kit can not be used.
- 2) PAGs are a class of protein produced by placenta or embryo, which means the level of PAG is highly related to the embryo viability. In early stage of pregnancy, embryo may stop to develop and abortion may occur, in such cases, the test result may be positive but due to the delay of PAG concentration change. That is on day 28 post AI, the test result may be positive and on day 32-35, test result may be negative.
- 3) In the field testing with cows on day 28 to day 34 after AI, serum or plasma samples demonstrated higher sensitivity for testing of pregnancy, at 32-34days post AI or mating, the sensitivity of pregnancy testing is over 99.5%.
- 4) In the field testing, the kit detects open cows more accurately at 28 days post AI with accuracy over 99.1%, which means all negative samples detected nonpregnant at 28 days with this kit will remain nonpregnant using ultrasound scanners at 32-35days post AI.
- 5) Based on the data obtained in field testing, it is recommended to set up customized reproduction program with your vet so that this kit work more practical in your farm. This method is not a replacement for ultrasound scanners, use it wisely to build a tool set for your farm reproduction program. For example, on day 28 post AI, use this kit to detect all non-pregnant cows and run AI. On day 32-35 use this kit to monitor fetus or embryo viability.



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## Quick Test Guide

1. Collect the blood from cow tail vein into EDTA blood tube.
2. Add blood into sample well on the test card.
3. Add sample buffer on the sample well.
4. Wait for 15min and check result.





**1 Collect blood sample**  
(From tail vein into collection tube)

**2 Add 1 drop of blood**  
(Into the sample well)

**3 Add 2 drops of buffer**  
(Into the same well)

**4 Wait 15 minutes**  
(Read result)

**RESULT:**

 • **2 lines = Pregnant** |  **1 line = Not pregnant**

Read the result within 15–20 minutes. Do not interpret the result after 20 minutes.



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