

## Bovine Pregnancy Rapid Test Kit

Art. No.: 200034-5T

Early diagnosis of pregnancy is of great importance in modern animal industry due to the high cost of feed and labor. Especially after artificial insemination is widely used, the earlier the pregnancy is determined, the earlier feeding plans will be performed. The general methodology to determine bovine pregnancy is by observation or ultrasound scanners, either of which is not quite economic in small or middle size farms. The current bovine pregnancy rapid test kit is a 15min lateral flow immunoassay kit that utilizes the high affinity of monoclonal antibody against bovine pregnancy associated glycoprotein (PAG), which can easily identify the concentration and presence of PAGs.

### 1. Application

This kit is for bovine pregnancy rapid testing in serum or plasma from 28 days after mating or AI. For whole blood sample, it is recommended test at 35days post AI. In addition, due to the long half-life of the target, testing is not recommended until 70 days after calving.

### 2. Kit components

- Rapid Test Card, 5pcs, in foil pouch
- Sample buffer, 5 tubes
- Needle and EDTA blood tube, 5 sets
- Pipette, 10pcs
- Kit instruction

**Caution:** the EDTA blood tube is only for blood collection. The tube expiry information does not compromise the performance of the test kit. It can be used within the shelf-life of the test kit.

### 3. Sample preparation

**Whole blood: Collect blood from cattle on day 35 or later post AI.**

Draw blood in EDTA blood tubes and mixed well by slowly inverting tube 10 times immediately after sample collection. The whole blood can be stored for less than 1 day at 2-8°C, whole blood cannot be frozen.

**For serum and plasma, collect sample from cattle on day 28 or later post AI.**

**Serum:** collect blood in tubes without anti-coagulant and processed to serum by centrifuge. For example, centrifuge at 5000rpm for 5min, then use the upper clear liquid as serum. Store at 2-8°C for no more than 1 day.

**Plasma:** collect blood in tubes containing EDTA (the blood tube in the kit) and mixed well by inverting immediately after sample draw. Tubes should be processed to plasma by centrifuge. For example, centrifuge at 5000rpm for 5min, then use the upper clear liquid as plasma. Store at 2-8°C for no more than 1 day.

### 4. Operations

- 1) Read the instruction before experiment. All samples shall be freshly collected. Bring the test kit and the test samples to room temperature (20-30°C).
- 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 (90-120µl)** 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.

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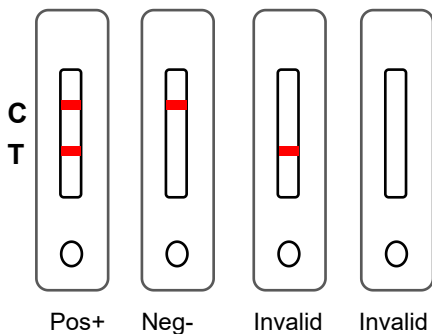
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- 4) Wait for 15min and then determine the result according to **Part 5**. Results observed after 30min is invalid.

## 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.