

Canine Pregnancy Test Kit

ReproCHEK™

For the
Detection of
the canine hormone
Relaxin

DIRECTION INSERT

ReproCHEK™ test results can be obtained in 15 minutes. The test kit contains a Positive Control and a Negative Control which must be included each time the test is performed. Visual comparison of the color of sample to the Negative Control will allow accurate detection of the presence of relaxin in the sample. If desired, test results may be determined by the use of a microwell plate reader.

III. SAMPLE INFORMATION

50 µL of plasma or whole blood is required. **Serum and EDTA anticoagulated samples must not be used.** Samples may be stored at 2°-7°C up to 48 hours. Plasma samples may be kept frozen for longer storage. Severely hemolyzed or lipemic serum may produce background color. When in doubt, obtain a better quality sample.

IV. PREPARATION OF WASH SOLUTION

Allow 10X Wash Concentrate to come to room temperature. Mix gently by inversion. Dilute wash concentrate 10-fold (1 part concentrate to 9 parts distilled water), mix and place in a wash bottle.

V. RESULTS

A. CONTROLS—For a valid test, the Positive Control must produce a distinct blue color and the Negative Control should be clear to light blue in color. If color does not develop in the Positive Control well or if there is distinct blue color development in the Negative Control well, results are invalid and the test should be repeated.

B. EVALUATION OF TEST WELLS in a valid test—

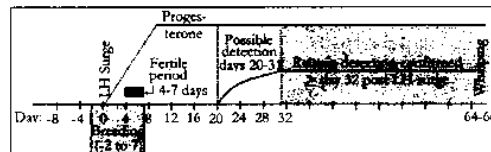
1. Samples producing distinct blue color are **POSITIVE** for relaxin.

I. INTRODUCTION

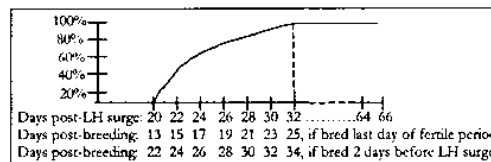
Synbiotics' ReproCHEK™ Canine Pregnancy Test Kit uses highly purified and specific antibodies to detect the presence of the hormone relaxin in canine **whole blood or plasma**. The presence of significant amounts of relaxin indicates pregnancy. The ReproCHEK™ test offers an early, inexpensive and reliable means of determining the success or failure of a planned mating or unwanted exposure. The ReproCHEK™ test may also be used to detect a sudden decrease in relaxin levels to indicate that spontaneous abortion has occurred.

ReproCHEK detects relaxin produced by the developing placenta(s) as early as day 20 after the luteinizing hormone (LH) surge. It is suspected that factors such as breed, size of the bitch, and litter size may have some influence on the level of serum relaxin. Although approximately 80% of pregnant bitches are detected between 20 and 28 days post-LH surge, some may not be detected as positive until day 31. Since canine sperm have fertile intrauterine lifespan of 6 to 7 days after breeding (Concannon et al: 1983), initial detection post-breeding may vary (see below). For example, a bitch detected positive for relaxin 22 days post-LH will be claimed positive 24 days post-breeding if bred 2 days before the LH surge, and 16 days post breeding if bred 6 days after the LH surge.

In general, if the bitch was bred at the appropriate time in her estrous cycle, pregnancy may first be determined between 20 and 31 post-LH surge, or, 14 and 33 days post-breeding.



PERCENT OF PREGNANCIES DETECTED



II. TEST PRINCIPLES

The ReproCHEK™ test kit is a microwell immunoassay for determining pregnancy in dogs. The plastic wells are coated with polyclonal anti-relaxin antibodies. A second antibody, highly specific to relaxin, is labeled with the enzyme horseradish peroxidase (HRP). The sample to be tested is placed directly into the coated well containing diluted HRP-antibody conjugate. Relaxin present in the sample will bind to the well and the HRP-antibody conjugate at the same time. The free HRP-antibody conjugate is washed away and a chromogenic substrate is added. The development of a blue color greater than the blue color of the negative control indicates the presence of relaxin in the sample. Absence of color indicates no relaxin in the sample.

2. Samples producing no color or blue color or less intensity than the blue color of the Negative Control are **NEGATIVE** for relaxin.
3. After the test is completed, the sample well can be detached and held alongside the Negative Control well against a white background for easier visual inspection.
4. If desired, results may be read on a microwell plate reader using an air blank. For single wavelength readers, set the wavelength of the plate reader at 630 nm. For plate readers with dual wavelength capability, set the test wavelength at 630 nm and reference wavelength at 450 nm or 490 nm. Samples producing an Optical Density equal to or less than the optical density of the Negative Control are **NEGATIVE** for relaxin. Samples producing an optical density greater than the optical density of the Negative Control are **POSITIVE** for relaxin.

VI. CONTENTS OF ReproCHEK™ TEST KIT

Antibody Coated Wells	48 wells
Bottle A - Conjugate Diluent	2.5 mL
Bottle B - Conjugate	2.5 mL
Bottle C - Negative Control	1.5 mL
Bottle D - Positive Control	1.5 mL
Bottle E - 10X Wash Solution	100 mL
Bottle F - Chromogenic Substrate	10 mL
Microwell Holder	
50 µL pipet; disposable pipet tips	
Additional material required:	
Deionized or distilled water	
Squirt bottles (2); Timer	
Optional: Microwell plate reader	

VII. PRECAUTIONS

1. Allow kit to come to room temperature (21°-25°C; 70°-78°F) prior to use.
2. Use separate pipet tip for each sample.
3. It is not recommended to test more than 10 samples per run.
4. Do not expose kit to direct sunlight.
5. Do not use expired reagents or mix from different kit lots.
6. Follow instructions exactly. Improper washing or contamination of reagents may produce non-specific color development.
7. **FOR VETERINARY USE ONLY.**

VIII. STORAGE AND STABILITY

Store the test kit and unused diluted wash solution at 2°-7°C (36°-45°F). Do not freeze. Reagents are stable until expiration date provided they have been stored properly.

FOR TECHNICAL ASSISTANCE:

1-800-228-4305



16420 Via Esprillo
San Diego, CA 92127

U.S. Veterinary License Number 312

03-1152-0303

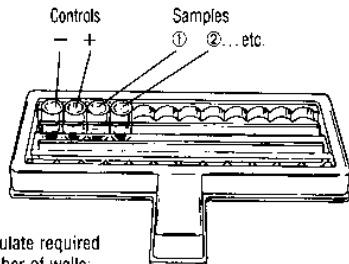
ReproCHEK™ Test Procedure

NOTE: Serum and EDTA anticoagulated samples must NOT be used.

Prior to use, allow kit components to come to room temperature (21°–25°C; 70°–78°F)

A. PREPARATION

1



- Calculate required number of wells:

- 1 well for Negative Control
- 1 well for Positive Control
- 1 well for each sample

NOTE: No more than 10 samples should be tested at one time.

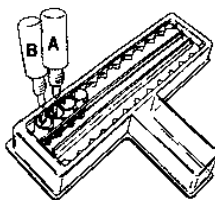
- Remove required number of wells.
- Leave wells attached to each other.
- Place wells in well holder.

NOTE: If a microwell plate reader will be used to read the results, leave the appropriate space empty so that the plate reader will blank on air.

B. CONJUGATE

2

- Add 1 drop Reagent A – Conjugate Diluent (White Cap) into each well.
- Add 1 drop Reagent B – Conjugate (Blue Cap) into each well.

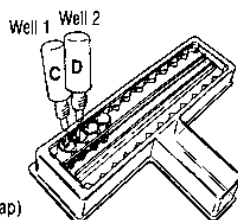


C. SAMPLE ADDITION

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CONTROLS

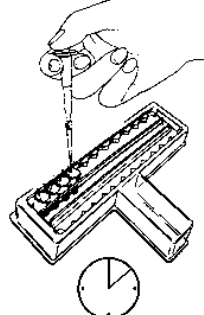
- Add 2 drops Bottle C – Negative Control (Grey Cap) into the first well.
- Add 2 drops Bottle D – Positive Control (Red Cap) into the second well.



SAMPLES

- Add 50µL of sample into the next well following the controls.
- For each additional sample, add 50µL to subsequent wells. Use a separate pipette tip for each sample.
- **WAIT 10 MINUTES**

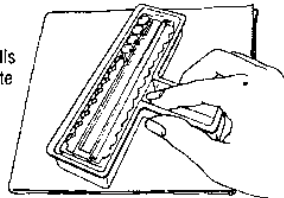
(TAP side of well holder for the first 15 seconds of the 10 minute incubation period. Be careful not to splash reagents.)



D. BLOT AND WASH

4

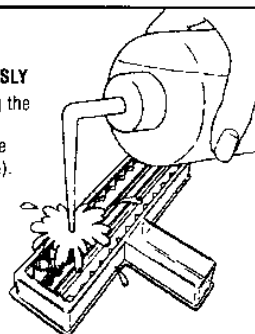
- Discard fluid from wells into sink or appropriate container.
- Invert holder and blot firmly onto a paper towel to remove final drops.



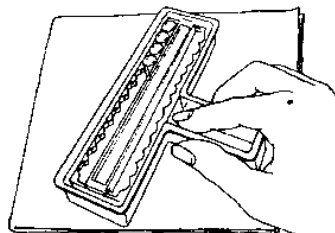
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FLUSH WELLS VIGOROUSLY

- Wash by vigorously filling the wells to overflowing with *diluted wash solution* (See Section IV for preparation).
- Direct a forceful stream into each well. (Oversplashing will not contaminate adjacent wells).
- Shake out excess wash solution.
- Repeat wash cycle **5 times**. Wash wells **2 more times** with distilled or deionized water to remove bubbles.



- Blot against a paper towel to dry wells.



E. DEVELOP

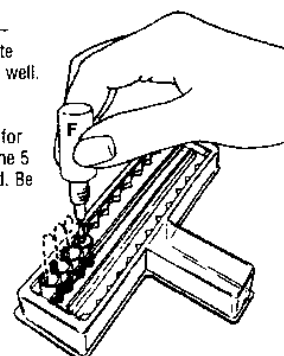
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- Add 3 drops Bottle F – Chromogenic Substrate (Green Cap) into each well.
 - **WAIT 5 MINUTES**
- (TAP side of well holder for the first 15 seconds of the 5 minute incubation period. Be careful not to splash reagents.)

5 minutes

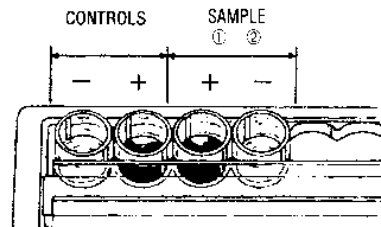


- **READ RESULTS AT EXACTLY 5 MINUTES.**



F. INTERPRETATION OF RESULTS

7



CONTROLS

- **NEGATIVE CONTROL** should be clear to light blue in color.
- **POSITIVE CONTROL** should be distinctly blue.

NOTE: If controls appear different than above the test is invalid and must be repeated.

SAMPLES

- **POSITIVE** samples will produce blue color darker than the color of the Negative Control. If a plate reader is used to read the results, please refer the Results section (section V), part B, number 4.
- **NEGATIVE** samples will produce no color or blue color of less than the blue color of the Negative Control. If a plate reader is used to read the results, please refer the Results section (section V), part B, number 4.

GOOD TECHNIQUES = ACCURATE RESULTS

- Plasma or anticoagulated whole blood may be used as a sample; however **DO NOT USE SERUM OR EDTA ANTICOAGULATED SAMPLES.**
- Hemolyzed and lipemic samples may be used; however, severely hemolyzed and/or lipemic samples may produce blue background color. When in doubt, obtain a better quality sample.
- **Washing is a very important step. Microwells cannot be overwashed.** Underwashing may result in non-specific blue color development in the negative control and sample wells.
- Prolonged incubation for more than 5 minutes in Step 6 may result in non-specific color development.
- Do not use the test kit past the expiration date and do not intermix components from different serial numbers.
- Store kit at 2°–7°C (36°–45° F). Allow kit to come to room temperature before use.