

TEST PRINCIPLES

DiroCHEK® is an enzyme-linked immunosorbent assay for the detection of adult *Dirofilaria immitis* antigen in canine and feline plasma or serum. The reaction wells are coated with antibodies directed against *D. immitis* antigen. Another antibody is labeled with the enzyme horseradish peroxidase. Any antigen present in the specimen (plasma or serum) is bound by the antibody coated well and the enzyme-linked antibody to form a specific complex. Any free enzyme-linked antibody is washed away and a chromogenic substrate is added. In the absence of *D. immitis* antigen, no color change will be observed. The development of a blue color specifically indicates the presence of *D. immitis* antigen.

CONTENTS

Anti- <i>D. immitis</i> Coated Wells.....	1056
Well Holder.....	1
Positive Control (Red Cap).....	3.5 ml
Negative Control (Gray Cap).....	3.5 ml
Reagent 1 - HRP Antibody Conjugate (Blue Cap).....	60 ml
Reagent 2 - Chromogenic Substrate Buffer (Purple Cap).....	2 x 60 ml

Materials required but not provided:
Precision pipet and tips
Distilled or deionized water

Materials suggested but not provided:
Multi-channel pipet
Reagent basins

Patent No. 4,789,631

PRECAUTIONS

1. Prior to use, allow kit to come to room temperature (21°-25°C; 70°-78°F).
2. To prevent contamination, do not return reagent to bottle.
3. Do not mix reagents between different serials.
4. Do not expose kit to direct sunlight.
5. Do not use expired components
6. Follow instructions carefully.
7. FOR LABORATORY USE ONLY.

STORAGE AND STABILITY

Store the test kit at 2°-7°C (36°-45°F). Do not freeze. Reagents will be stable until the expiration date provided they have been stored properly.

SAMPLE INFORMATION


50 µl (0.05 ml) of serum or plasma is required. Serum or plasma (not whole blood) may be stored at 2°-7°C (36°-45°F) for 7 days; -20°C or below if stored longer. Severely hemolyzed and/or lipemic samples may produce background color. When in doubt, obtain a better quality sample.

PREDICTING HEARTWORM BURDEN

Level of color in sample wells will intensify with increasing level of heartworm antigen. Level of heartworm antigen correlates with the number of heartworms present, although it is more closely related to the actual weight of worms present (a product of both the number and size of worms).*

*Courtney CH, Zeng Q, Bean ES. Predicting Heartworm Burdens with the DiroCHEK Heartworm Antigen Test Kit. J Am Anim Hosp Assoc 1989;25:643-646

FOR TECHNICAL ASSISTANCE: 1-800-228-4305

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Canine Heartworm Antigen Test Kit

DiroCHEK® KILO PACK

For the
Detection of
Canine and Feline
Heartworm
Infection

DIRECTION INSERT

DiroCHEK[®] Heartworm Test Procedure

NOTE: Use PLASMA or SERUM samples only (NO WHOLE BLOOD).
Prior to use, allow kit components to come to room temperature (70°-78° F; 21°-25° C).

TEST PROCEDURE

- Step 1:** Determine required number of antibody coated wells. One well for the Positive Control, 1 well for the Negative Control and 1 well for each sample. Remove required number of plates and/or removable wells. Place removable wells in the well holder, leaving wells attached to each other.
- Step 2:** Place 1 drop or 0.05 mL of **Positive Control** into the first well. Place 1 drop or 0.05 mL of **Negative Control** into the second well. Place 0.05 mL of sample into the next well. Using a separate pipet tip for each sample, place 0.05 mL of each additional sample into subsequent wells.
- Step 3:** Add 0.05 mL of **Reagent 1** (Conjugate) into each well. Tap well holder (without splashing) for 15 seconds to mix.

WAIT 10 MINUTES.

- Step 4:** Discard fluid in wells into sink or appropriate container. Invert and blot firmly against a paper towel.
- Step 5:** **Wash wells vigorously** by directing a forceful stream of distilled or deionized water into each well. (Oversplashing will not contaminate adjacent wells.) Shake out residual liquid. Repeat wash at least 5 separate times. **Wells cannot be overwashed.** Invert and blot against a paper towel to remove final drops of water.
- Step 6:** Add 0.10 mL of **Reagent 2** (Chromogenic Substrate Buffer) to each well. Tap well holder (without splashing) for 15 seconds to mix.

WAIT 5 MINUTES. Read results.

INTERPRETATION OF RESULTS

- Controls:** For the test to be valid, the **Positive Control** should be distinctly blue and the **Negative Control** should be clear.
- Samples:** **Positive** samples will be blue. Color intensity will vary with level of heartworm antigen present. This may reflect worm burden. (See PREDICTING HEARTWORM BURDEN on back page.)
- Negative** samples will be clear.

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