**CANINE PREGNANCY TEST KIT**

**WITNESS’ RELAXIN**

**GENERAL INFORMATION**

The WITNESS’ RELAXIN kit is intended to determine pregnancy in the bitch by measuring relaxin levels in plasma and serum samples. The presence of significant amounts of this hormone is a reliable indicator of pregnancy. A sudden decrease in relaxin may indicate that abortion has occurred. The WITNESS RELAXIN kit 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 influences 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 a fertile period of 4 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 the breeding occurred 6 days after.

**PERCENT OF PREGNANCIES DETECTED**

<table>
<thead>
<tr>
<th>Days post-LH surge</th>
<th>Positive results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>70</td>
</tr>
<tr>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>19</td>
<td>100</td>
</tr>
</tbody>
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**TEST PRINCIPLE**

WITNESS RELAXIN is a simple test based on Rapid Immuno-Magnahion (RIM) technology, using the combination of anti-canine relaxin antibodies to quickly identify this hormone in biological samples (serum or plasma) from the bitch. Sensitive colloidal gold particles bind to relaxin molecules present in the sample. The complexes migrate along a nitrocellulose strip and are then captured on a sensitised reaction line where its accumulation causes the formation of a clearly visible pink/purple band. A control band, located at the end of the reading window, ensures that the test was performed correctly.

**SAMPLE INFORMATION**

- The test can be performed on serum or plasma (pre-anticoagulated with EDTA, sodium citrate or heparin). Do not use whole blood.
- Sample should always be collected with a sterile needle and syringe.
- Hemolysis does not significantly interfere with the test, but strongly hemolyzed samples may partly obscure a weak positive line (due to hemoglobin background).

**SAMPLE STORAGE**

It is recommended to test samples immediately after collection. If samples are kept at room temperature, they should be tested within 4 hours. If testing is further delayed, samples should be refrigerated at 2-8°C (35-45°F) and may be held for up to 48 hours.

**KIT CONTENTS**

- 5 pouches, containing 1 test device and desiccant
- 1 Buffer dropper bottle (2 ml)
- Instructions for use
- 5 pipettes

**GENERAL PRECAUTIONS**

- Do not use components after expiration date.
- Refrigeration not required. Store the test kit at 2-25°C (35-77°F).
- Do not freeze.
- Use the test within 10 minutes after opening the sealed pouch.
- Avoid touching or damaging membrane at windows #1, #2, #3.
- The WITNESS device should be placed on a flat, horizontal surface while performing the test.
- Use a separate pipette for each sample.
- Hold pipette and buffer bottle vertically when dispensing.
- For veterinary use only.

**TEST PROCEDURE AND RESULTS**

**Important:** Allow sample and buffer drops to fall onto membranes at window #1. Do not touch pipette tip, sample or buffer drops, or buffer bottle tip directly to the membrane.

1. **Sample application**
   - Tear open the pouch provided and place the test device on a flat horizontal surface for the duration of the test.
   - Squeeze the provided pipette near the sealed end. Insert the end of the pipette into a serum or plasma sample. Release the pressure slightly to draw up a small amount of sample into the pipette.
   - Holding the pipette vertically, transfer two drops of sample to the sample well, window #1.

2. **Buffer dispensing**
   - Remove the cap from the buffer bottle, hold it vertically and add two drops of buffer to the sample well window #1.

3. **Reading test**
   - Wait ten minutes. Observe the presence or absence of pink/purple bands in reading windows #2 and #3.
   - Sample results are read in window #2. The control band is read in window #3.

**Notes:**
- The test is complete and may be read before 10 minutes if pink/purple bands are visible in both windows #2 and #3.
- The presence of a pink/purple band only in window #3 before 10 minutes does not mean that the test is complete. A pink/purple band in window #2 may develop slower than the control pink/purple band in window #3.

4. **Results Validation**
   - Valid test: Test is validated if a pink/purple band is present in the reading window #3.
   - Negative for relaxin: No bands in reading window #2, with one pink/purple band in window #3.
   - Positive for relaxin: One pink/purple band in reading window #2, with one pink/purple band in window #3.

**Note:** For more information consult GENERAL INFORMATION section and tables on front page of insert.

- Negative Samples: Any negative result is considered indicative of non-pregnancy; however, two negative results one week apart may be required for confirmation of this status, especially when date of mating is unknown.
- Positive Samples: A positive result is considered indicative of pregnancy. A weak positive result may be observed at the beginning of the pregnancy. It is recommended to repeat the test on a fresh sample one week later to confirm pregnancy.

**Invalid Test**

- Invalid test: No pink/purple band in control window #3.

**Note:** A test result should always be interpreted in the context of all available clinical information and history for the dog.