Product Description

The Canine Fentanyl Direct ELISA Kit is a sensitive in vitro test to detect the presence of fentanyl in samples such as whole blood, serum, plasma, and urine. Fentanyl is a synthetic narcotic analgesic of high potency and short duration of action. Though 200 times more potent than morphine, fentanyl has a high safety margin. While Fentanyl has all the properties of morphine, it is structurally different and therefore, cannot be detected by screening tests for morphine and related opiates. Because of the potency of the drug, concentrations encountered in biological fluids are in the subnanogram range.

The Canine Fentanyl Direct ELISA Kit is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture. A 20 µL aliquot of a diluted unknown specimen is incubated with a 100 µL dilution of enzyme (horseradish peroxidase) labeled fentanyl derivative in microplate wells, coated with fixed amounts of high affinity purified polyclonal anti-fentanyl. The wells are washed thoroughly and a chromogenic substrate added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample. The technique is sensitive to 0.1 ng/mL.

The Canine Fentanyl Direct ELISA Kit avoids extraction of urine or blood samples for measurement. It employs a fentanyl directed antiserum. Due to the proprietary method of orienting the antibody on the polystyrene microplate much higher sensitivity is achieved compared to passive adsorption. This allows an extremely small sample size, reducing matrix effects and interference with binding protein(s) or other macromolecules.

Components

<table>
<thead>
<tr>
<th>Materials provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells with polyclonal anti-Fentanyl</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Fentanyl Conjugate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Immunalysis Positive Reference Standard</td>
<td>2 mL</td>
</tr>
<tr>
<td>Negative Standard</td>
<td>1 mL</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Reagent</td>
<td>12 mL</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water,
2. Precision pipettes.
3. Disposable pipette tips.
4. ELISA reader capable of reading absorbance at 450 nm.
5. Absorbent paper or paper towel.
6. Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
**Preparation Instructions**

**Sample Preparation**
1. The Canine Fentanyl Direct ELISA Kit is to be used with canine samples, such as whole blood, oral fluids, serum, plasma, and urine. All possible applications of this assay have not been tested.
2. Specimens to which sodium azide has been added affect the assay.
3. Urine samples should be stored at 2–4 °C until use. Samples should be well mixed before assay. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with ice packs or equivalent.

**Storage/Stability**
Store the kit at 2–8 °C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose test reagents to heat, sun or strong light.

**Procedure**

**Notes:** The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that serum samples be run in duplicate.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

All reagents must be brought to room temperature before use. The procedure as described may be followed in sequence using manual pipettes. Alternatively all reagents may be added using an automated pipettor.

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1. Dilute specimens, to the necessary range with Phosphate Buffered Saline, pH 7.0–7.4. Urine samples using a 0.5 ng/mL (500 pg/mL) cutoff do not require dilution. The dilution factor can be adjusted based on the laboratory’s cutoff.
2. Add 20 µL of positive standards and negative standards into appropriate wells in duplicate.
3. Add 20 µL of the diluted specimens in duplicate (recommended) to each well.
4. For postmortem samples, add 100 µL of 100 mM Phosphate Buffered Saline to each well. (Optional)
5. Add 100 µL of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
6. Incubate for 60 minutes at room temperature (18–26 °C) preferably in the dark, after addition of enzyme conjugate to the last well.
7. Wash the wells 6 times with 350 µL of distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples containing abnormally high amounts of hemoglobin (some postmortem samples), use 10 mM Phosphate Buffered Saline, pH 7.0–7.4. This will lower potential non-specific binding of hemoglobin to the well, thus lowering background color.
8. Invert wells and vigorously slap dry on absorbent paper to ensure all residual moisture is removed. This step is critical to ensure that residual enzyme conjugate does not skew results. If using an automated system, ensure that the final aspiration on the wash cycle aspirates from either side of the well.
9. Add 100 µL of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
10. Incubate for 30 minutes at room temperature, preferably in the dark.
11. Add 100 µL of Stop Solution to each well, to change the blue color to yellow.
12. Measure the absorbance at a dual wavelength of 450 nm and 650 nm. Compare average absorbance readings obtained from each unknown specimen with the average absorbance obtained from the Positive Reference Standard.
13. Wells should be read within one hour of yellow color development.
Results
The standard curve is constructed as follows:
1. To construct the standard curve, plot the absorbance for fentanyl standards (vertical axis) versus fentanyl standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
2. Read the absorbance for the controls and each unknown sample from the curve. Record the value for each control and unknown sample.

Typical Data for Standard Curve
The following data represent a typical dose/response curve.
Note: These data were obtained by diluting the positive standard with Phosphate Buffered Saline, pH 7.0–7.4.

<table>
<thead>
<tr>
<th>Standards</th>
<th>OD (450 nm)</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>2.100</td>
<td>0</td>
</tr>
<tr>
<td>Std 2</td>
<td>1.384</td>
<td>0.1</td>
</tr>
<tr>
<td>Std 3</td>
<td>0.733</td>
<td>0.5</td>
</tr>
<tr>
<td>Std 4</td>
<td>0.561</td>
<td>1.0</td>
</tr>
<tr>
<td>Std 5</td>
<td>0.239</td>
<td>5.0</td>
</tr>
</tbody>
</table>

The data shown should not be used in assay calculations. It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve or cutoff standards should be run with every plate.

Reference
1. Baselt, R.C., and Cravey, R.H., Disposition of Toxic Drugs and Chemicals in Man. Chemical Toxicology Institute, Foster City 319-321.