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Product Information

Canine Fentanyl Direct ELISA kit

Catalog Number: EA100904
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Canine Fentanyl Direct ELISA Kit provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/ mass spectrometry (GS-MS) is the preferred confirmatory method. Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Background

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. The drug is available as a citrate salt in an injectable solution containing 50 µg/ml. It is also available as a transdermal patch containing 2.5 – 10 mg Fentanyl and provides a dose of 25 –100 µg/hr for 72 hours for management of chronic pain (1). 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 sub nanogram range (2).

Principle of the Test

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 micro-plate 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 sample for measurement. It employs a Fentanyl directed antiserum. Due to the proprietary method of orienting the antibody on the polystyrene micro-plate much higher sensitivity is achieved compared to passive adsorption. This allows an extremely small sample size, reducing matrix effects and interference with binding proteins(s) or other macromolecules.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell with polyclonal anti-Fentanyl	12x8x1

2. Fentanyl- Conjugate	12 ml
3. Fentanyl Positive Ref. Std (5 ng/mL)	2 ml
4. Negative Standard	1 ml
5. TMB Substrate	12 ml
6. Stop Reagent	12 ml

Materials 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 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation

1. The Fentanyl specific Direct ELISA Kit is to be used with Canine samples, such as whole blood, oral fluids, serum, plasma and urine. OriGene has not tested all possible applications of this assay.
2. 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 Blue Ice or equivalent.

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- 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 standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Dilute specimens, to the necessary range with Phosphate Buffer Saline pH 7.0-7.4. Urine samples using a 0.5 ng/ml cutoff do not require to be diluted. 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 post mortem samples, add 100 µl of 100 mM Phosphate Buffer 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 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 nonspecific 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.

Calculations of Results

The standard curve is constructed as follows:

1. Check Fentanyl standard value on each standard vial. To construct the standard curve, plot the absorbance for Cotinine standards (vertical axis) versus Cotinine standard concentrations (horizontal axis). Draw the best curve through the points.
2. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

The following data represent a typical dose/response curve.

Standards	Conc. (ng/ml)	Absorbance (450nm)
STD1	0	2.100
STD2	0.1	1.384
STD3	0.5	0.733
STD4	1.0	0.561
STD5	5.0	0.239

Note: this curve was obtained by diluting the positive standard with Phosphate Buffer Saline PH 7.0-7.4

The dose/response curve shown above 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.

Sensitivity

Assay sensitivity based on the minimum Benzoylecgonine concentration required to produce a four standard deviation from assay zero dose response (A_0) is 0.1 ng/ml.

References

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

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