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Product Information

Canine Morphine Specific Direct ELISA kit

Catalog Number: EA100909
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Morphine Specific 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. The Morphine Specific Direct ELISA Kit is a specific and sensitive in-vitro test to detect the presence of Morphine in samples such as whole blood, serum, plasma and urine.

Principle of the Test

The Morphine Specific 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 morphine derivative in micro- plate wells, coated with fixed amounts of oriented high affinity purified polyclonal antibody. 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 1 ng/ml. The Morphine Specific Direct ELISA Kit avoids extraction of urine sample for measurement. It employs a Morphine Specific 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. Microwells coated with polyclonal anti-morphine	12X8X1
2. Morphine-Conjugate	12.5 ml
3. Immunalysis Positive Reference Standards	1 ml
4. Negative Standards	1 ml
5. TMB substrate	14 ml
6. Stop Reagent	12.5 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 Morphine specific Direct ELISA Kit is to be used with human samples, such as urine, whole blood, oral fluids, serum and plasma. OriGene has not tested all possible applications of this assay. Cutoff criteria are important in deciding the sample dilution.
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. (Urine samples are normally diluted 1:10 for a cutoff level of 300 ng/ml of morphine.) The dilution factor can be adjusted based on the laboratory cutoff.
2. Add 20 µl of calibrators and standards to each well in duplicate.
3. Add 20 µl of the diluted specimens in duplicate (recommended) to each well.
4. Add 100 µl of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
5. Incubate for 60 minutes at room temperature preferably in the dark at room temperature (20-25°C), after addition of enzyme conjugate to the last well.
6. Wash wells 6 times with 350 ul 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.
7. 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.
8. Add 100 µl of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
9. Incubate for 30 minutes at room temperature (20-25°C), preferably in the dark.
10. Add 100 µl of Stop Solution to each well, to change the blue color to yellow.
11. 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.
12. Wells should be read within 1 hour of yellow color development.

Example of a Standard Curve

The following data represent a typical dose/response curve.

Standards	Morphine (ng/mL)	Absorbance (450nm)
STD1	0	1.910
STD2	5	1.624
STD3	10	1.457
STD4	25	1.241

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.

References

1. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph, 73,1986.
2. Drugs on the Job. Time Magazine, March 17, 1986
3. E.L.Way and T.K.Adler. Bull. Wld. Hlth. Org. 27:359 (1962)
4. R.C. Baselt. In : Advances in Analytical Technology, Vol.1. Randall C. Baselt edd. (Biomedical Publications, Foster City, CA. 112- 116).

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