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Cat#: OP092D (96 Tests)
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Opiates ELISA

Catalog No. OP092D (96 Tests)

INTENDED USE

The Opiates 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 (1). Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used. For research use only.

SUMMARY EXPLANATION

The Opiates Direct ELISA Kit is a specific and sensitive in-vitro test to detect the presence of Opiates in samples such as whole blood, oral fluids, serum, plasma and urine.
Heroin/morphine abuse is a major problem in society (2). In the body, both heroin (diacetylmorphine) and morphine are largely converted to morphine-3-glucuronide (MG)(3). The Opiates ELISA Kit measures heroin, morphine, codeine, hydrocodone and their metabolites.

PRINCIPLES OF THE TEST

The Opiates Direct ELISA Kit (for morphine equivalents measurement) 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 10 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 0.25 ng/mL. The Opiates Direct ELISA Kit avoids extraction of urine sample for measurement. It employs an Opiates 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.

MATERIALS PROVIDED	96 Tests
Microwells coated with polyclonal anti-morphine	12X8X1
Morphine-Conjugate	12 ml
Immunalysis Positive Ref. Std	2 ml
Neg Std	1 ml
TMB substrate	12 ml
Stop Reagent	11 ml

MATERIALS 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
6. Graph paper

STORAGE AND STABILITY

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

WARNINGS AND PRECAUTIONS

1. Not for Internal or External Use in Humans or Animals. There should be no eating or drinking within work area. Always wear gloves and a protective lab coat.
2. This kit is designed for Research Use Only.
3. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous. Do not add sodium azide to samples as preservative. Do not use external controls containing sodium azide. Bring all reagents to room temperature.
4. Use disposable pipet tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue. Do not pour chromogenic substrate back into container after use.
5. Do not freeze reagents. Do not mix reagents from different kit lot numbers.
6. Keep reagents out of direct sunlight. Handle stop reagent with care, since it is corrosive.
7. Viscous samples should always be diluted in phosphate buffered saline or distilled water prior to pippetting. Ensure the bag containing the micro-plate strips and dessicant is well sealed if only a few strips are used.

SPECIMEN COLLECTION

1. The Opiates Direct ELISA Kit is to be used with human samples, such as urine, whole blood, oral fluids, serum and plasma. has not tested all possible applications of this assay. Cutoff criteria are important in deciding the sample dilution.
2. Specimens to which sodium azide has been added affect the assay.

STORAGE AND HANDLING

1. Urine samples should be stored at 2 -4^0 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.
2. The expiration date of the kit is stated on the label.
3. The kit can be expected to perform satisfactorily until the expiration date if stored in the refrigerator at 2 – 4^0 C.

ASSAY PROCEDURE.

All reagents must be brought to room temperature (18-26^0 C) before use. The procedure as described below may be followed in sequence using manual pipettes. Alternatively all reagents may be added using an automated pipettor.

1. Dilute specimens, to the necessary range with Phosphate Buffer Saline pH 7.0. (urine samples are normally diluted 1:20 for a cutoff level of 300 ng/mL of morphine.) The dilution factor can be adjusted based on the laboratory's cutoff.
2. Add 10 µl. of appropriately diluted calibrators and standards to each well in duplicate.
3. Add 10 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 (18-26^0 C) preferably in the dark, after addition of enzyme conjugate to the last well.
6. 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.
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, 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.
12. Wells should be read within 1 hour of yellow color development

The following data represent a typical dose/response curve.

Morphine ng/mL	Absorbance
0	2.669
5	1.238
10	0.794
25	0.133

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 a cutoff calibrator should be run with every plate.

RESULTS

If the average sample absorbance is equal to or less than the average absorbance of the laboratory morphine positive reference standard the sample is <u>POSITIVE</u> for Opiates. If the average sample absorbance is greater than the average absorbance of the laboratory morphine positive reference standard the sample is called <u>NEGATIVE</u> for Opiates. Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding absorbance (ordinate). Values for unknown samples are obtained by interpolation from the curve.