Morphine ELISA Kit

Catalog Number KA0935

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

The Morphine 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.
This kit has been configured for research use only and is not for diagnostic and clinical use.

The Morphine 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.

Principle of the Assay

The Morphine 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 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.

General Information

Materials Supplied

List of component

MATERIALS PROVIDED	96 Tests
Microwells with polyclonal anti-Morphine	12x8x1
Morphine-Conjugate	12 mL
Immunalysis Positive Reference Standard	2 mL
Negative Standard	1 mL
TMB Substrate	12 mL
Stop Reagent	11 mL

Storage Instruction

- ✓ 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.

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

- Precautions
- ✓ Potential biohazardous materials:

  The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as

recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.

- ✓ This test kit is designed for Research Use Only.
- ✓ Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- ✓ 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.

Assay Protocol

Sample Preparation

- ✓ The Morphine ELISA Kit is to be used with human samples, such as whole blood, oral fluids, serum, plasma and urine. Has not tested all possible applications of this assay.
- ✓ Specimens to which sodium azide has been added affect the assay.
- ✓ 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

Bring all specimens and kit reagents to room temperature (18-26°C) and gently mix.

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 standards to each well in duplicate.
3. Add 20 µL of the diluted specimens in duplicate (recommended) to designated wells.
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 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 (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.

Data Analysis

Calculation of Results

The following data represent a typical dose/response curve.

Morphine ng/mL	Absorbance
0	1.910
5	1.624
10	1.457
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 a cutoff calibrator should be run with every plate.

Performance Characteristics

- Accuracy

50 whole blood samples and 40 urine samples collected from presumed non-users were tested u\in the Morphine ELISA KIT. One hundred percent of these normal sample measured negative at 25 ng/mL for whole blood and 300 ng/mL for urine. Fifty whole blood samples which were previously confirmed positive for Morphine by GC-MS employing a cut-off 25 ng/mL, were tested in the Morphine ELISA KIT. All of the samples were found to be positive i.e. above the cut-off of 25 ng/mL.

- Precision

The precision of the Morphine ELISA KIT has been verified by assessment of the mean, standard deviation (SD) and coefficients of variation (CV) in data resulting from repetitive assays.

Intra-assay precision was determined with reference controls. A 0.5, 10 and 25 ng/mL standard was assayed five times in the same assay. The results are tabulated in Table 1.

Morphine ng/mL	Mean Abs	S.D.	C.V.%
0	1.642	0.156	9.5
5	1.198	0.144	12.0
10	0.806	0.076	9.43
25	0.522	0.067	12.83

- Sensitivity

Assay sensitivity based on the minimum morphine concentration required to produce a four standard deviation from assay Ao is is 1 ng/mL.

- Specificity

The sensitivity of the ELISA for Morphine Specific was determined by generating inhibition curves for each of the compounds listed below:

Compound	Approx.ng/mL equivalent to morphine	Cross-reactivites
Morphine	25	100
Codeine	750	3.3
Morphine 3-glucuronide	1000	2.5
Morphine 6-glucoronide	2500	1
Ethyl morphine HCL	2500	1
6-acetyl-morphine	1250	2
Thebaine	1250	2
Meperdine HCL	2500	1
Hydromorphone HCL	2500	1
Oxycodone HCL	2500	1
Hydrocodone	2500	1
Hydromorphone	1250	2
Noroxycodone	2500	1
Noroxymorphone	2500	1

- Cross-Reactivity with Unrelated Drugs

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 2,500 ng/mL. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level (1 ng/mL). Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid Atropine, Barbital, Benzoylecgonine, Butabarbital, Caffeine, Cocaine, Carbamazepine, Chloroquine, Chloropromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5-Diphenylhydanoin, 10-11-Dihydrocarbamazipine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, MDA, MDMA, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Metharbital, Mephenytion, a-Methyl-a-propylsuccinimide, Mephobarbital, Methal PEMA, Methsuximide, 4-Methylprimidone, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOH.

Resources

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).

Plate Layout

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