

Product Information

Opiates ELISA

Catalog Number **SE120100**
Storage Temperature 2–8° C

TECHNICAL BULLETIN

Product Description

The Opiates ELISA 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 measures heroin, morphine, codeine, hydrocodone, and their metabolites.

The Opiates ELISA (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 microplate 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 ELISA avoids extraction of urine samples for measurement. It employs an opiates 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.

The Opiates ELISA 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.

Components

Materials Provided	96 Tests
Microwells coated with polyclonal anti-morphine	12 x 8 x 1
Morphine-Conjugate	12 mL
Immunalysis Positive Ref. Std	2 mL
Neg Std	1 mL
TMB substrate	12 mL
Stop Reagent	11 mL

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

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

All reagents must be brought to room temperature (18–26 °C) before use. The procedure as described 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 Buffered Saline, pH 7.0. (Urine samples are normally diluted 21-fold 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 °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

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 POSITIVE for Opiates.

If the average sample absorbance is greater than the average absorbance of the laboratory morphine positive reference standard the sample is called NEGATIVE 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.

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

References

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

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