Barbiturate ELISA Kit

Catalog Number KA0927

96 assays

Version: 02

Intended for research use only

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Introduction and Background

A. Overview

The Barbiturate ELISA Kit is a specific and sensitive in-vitro test to detect the presence of barbiturates in samples such as whole blood, serum, plasma and urine. Barbiturates - derivatives of Barbituric acid - are sedative drugs which at low doses induce relaxation and at high doses induce coma and even death (2). Barbiturates are usually administered orally but may also be taken intravenously or intramuscularly and are absorbed rapidly. The metabolism of Barbiturates is mainly in the liver, a number of metabolic pathways have been described which include oxidation, desulfuration and ring cleavage. Because the number and the proportion of the various Barbiturate metabolites varies with each individual the results are expressed in terms of the equivalents of the standard, secobarbital/ml.

B. Test Principle

The Barbiturate 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 10 µl aliquot of a diluted unknown specimen is incubated with a 100 µl dilution of enzyme (Horseradish peroxidase) labeled barbiturate 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 Barbiturate Direct ELISA Kit avoids extraction of urine or blood sample for measurement. It employs a polyclonal high affinity, purified Barbiturate antibody. Due to the proprietary method of orienting the antibody on the polystyrene micro-plate much higher sensitivity is achieved compared to passive adsorption. This results in extremely small sample size reducing matrix effects and interference with binding proteins(s) or other macromolecules.

C. Notice for Application of Kit

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

D. Intended Use

The Barbiturate ELISA Kit provides only a preliminary analytical test result. A more specific alternate chemical method must be used 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.

Material and Method

A. List of component

	96 Tests
Microwells with polyclonal anti-barbiturate	12x8x1
Barbiturate-Conjugate	12 ml
Immunalysis Positive Reference Standard	2 ml
Negative Standard	1 ml
TMB Substrate	12 ml
Stop Reagent	11 ml

B. Additional Required Materials 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
6. Graph paper

C. Stability and Storage

1. Store the kit at 2-8℃.
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.

D. Warnings and Precautions for Users

1. 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.
2. This test kit is designed for Research Use Only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. It is recommended that serum samples be run in duplicate.
6. 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.

E. Specimen Collection and Handling

1. The Barbiturate ELISA Kit is to be used with human samples, such as whole blood, serum, plasma and urine. Has not tested all possible applications of this assay.
2. Specimens to which sodium azide has been added affect the assay.
3. Urine samples should be stored at 2-4℃ 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.

F. Protocol

All reagents must be brought to room temperature (18-26℃) 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 secobarbital cutoff of 200 ng/ml.) The dilution factor and volume added 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℃) 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.

Example of a standard curve

The following data represent a typical dose/response curve.

Secobarbital ng/ml	Absorbance
0	1.955
5	0.758
10	0.652
25	0.514

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 Characteristic

1. Accuracy

45 whole blood samples and 50 urine samples collected from presumed non-users were tested in the Barbiturate ELISA Kit. One hundred percent of these normal samples measured negative at 50 ng/ml of secobarbital equivalents for whole blood and 200 ng/ml of secobarbital equivalents for urine. Thirty five whole blood samples which were previously confirmed positive for barbiturates by GC-MS employing a cut-off of 50 ng/ml equivalents, were tested in the Barbiturate ELISA Kit. All the samples were found to be positive i.e. above the cut-off of 50 ng/ml.

2. Precision

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

3. Intra-Assay Precision

Intra-Assay Precision was determined with reference controls. A 0, 5, 10 and 25 ng/ml standard was assayed eight times in the same assay. The results are tabulated in Table 1.

Secobarbital ng/ml	Mean Abs	S.D.	C. V. %
0	1.944	0.149	7.67
5	0.822	0.073	8.9
10	0.695	0.057	8.2
25	0.562	0.066	11.7

4. Sensitivity

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

5. Specificity

The specificity of the Barbiturate ELISA was determined by generating inhibition curves for each of the compounds listed below.

Compound	Approx. ng/ml equivalent to 25 ng secobarbital	Cross-reactivities
Aprobarbital	45	55
Butabarbital	60	42
Barbital	125	20
Amobarbital	48	52
Butalbital	55	46
p-Hydroxyphen-barbital	60	42
Pentobarbital	52	48
Diallylbarbituric acid	60	42
Phenobarbital	114	23
Barbituric Acid	>1250	<1
Hexobarbital	>1250	<1
Mephobarbital	>1250	<1

6. Cross-Reactivity with Unrelated Drugs

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 2000 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, Ascorbic acid, Atropine, Benzoylecgonine, Caffeine, Cocaine, Carbamazepine, Codeine, Chloroquine, Chloropromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5-Diphenylhydantoin, 10-11-Dihydro-carbamazepine, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Mephenytoin, "-Methyl-"-propylsuccinimide, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phensuximide, PEMA, Primidone, Phencyclidine, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, THC-COOH.

References

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3. R.C. Baselt. In: Advances in Analytical Technology, Vol.1. Randall C. Baselt ed. (Biomedical Publications, Foster City, CA. 93-97).