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Product Information

Barbiturate Direct ELISA Kit

Catalog Number **SE120148**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Barbiturate Direct 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 vary with each individual the results are expressed in terms of equivalents of the standard (secobarbital)/mL.

The Barbiturate 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 10 μL aliquot of a diluted unknown specimen is incubated with a 100 μL dilution of enzyme (Horseradish peroxidase) labeled barbiturate 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 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 microplate much higher sensitivity is achieved compared to passive adsorption. This results in extremely small sample size reducing matrix effects and interference with binding protein(s) or other macromolecules.

The Barbiturate 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.

Components

Materials Provided	96 Tests
Microwell with polyclonal anti-barbiturate	12 x 8 x 1
Barbiturate-Conjugate	12 mL
Immunalysis Positive Reference Standard	2 mL
Negative Standard	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 Barbiturate Direct ELISA kit is to be used with human samples, such as whole blood, oral fluids, serum, plasma, and urine. All possible applications of this assay have not been tested.
2. Specimens to which sodium azide has been added affect the assay.
3. 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 ice packs or equivalent.

Storage/Stability

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

Procedure

Notes: 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.

All reagents must be brought to room temperature (18–26 °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 buffered 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 appropriate wells in duplicate.
3. Add 10 µL of the diluted specimens in duplicate (recommended) to appropriate 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 (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 of 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 non-specific 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

The dose/response curve 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.

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

References

1. Weiner, N., Norepinephrine, epinephrine and the sympathomimetic amines. In: The Pharmacological Basis of Therapeutics. 7th ed. p.145-180 (New York: MacMillan 1985).
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3. Caldwell, J., and Sever, P.S., The Biochemical Pharmacology of Abused Drugs. Clinical Pharmacology and Therapeutics. 16: 625- 638 (1974).
4. Baselt, R.C., in : Advances in Analytical Technology, Vol.1. Randall C. Baselt ed., Biomedical Publications, (Foster City, CA) 87-93.

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