



Barbiturate Direct ELISA Kit

Catalog No. BA081D (96 tests)

INTENDED USE

The Calbiotech 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.

SUMMARY AND EXPLANATION

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.


PRINCIPLE OF THE TEST




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 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.

MATERIALS PROVIDED	96 tests
1. Microwells coated with polyclonal anti-barbiturate	12x8x1
2. Barbiturate Conjugate, 1 bottle	12.5 ml
3. Barbiturate Standard, 3 vials	1 ml
4. Negative Control, 1 vials	1 ml
5. TMB Substrate, 1 bottle	14ml
6. Stop Reagent, 1 bottle	12.5ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Absorbance paper or paper towel
5. Graph paper

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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. 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. Not for use in diagnostic procedures.
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.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

ASSAY PROCEDURE

All reagents must be brought to room temperature (18-26° C) before use.

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° 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.

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.

REFERENCES

1. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph, 73, 1986.
2. S.C. Harvey, In: The Pharmacological Basis of Therapeutics, 5th Ed., L.S. Goodman and A. Gilman edd. (New York, Macmillan, 1975) (pp102-23)
3. R.C. Baselt. In: Advances in Analytical Technology, Vol.1. Randall C. Baselt ed. (Biomedical Publications, Foster City, CA. 93-97).

For Research Use Only. Not for use in Diagnostic Procedures.