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Product Information

Benzodiazepines Direct ELISA

Catalog Number **SE120149**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Benzodiazepines are a class of widely prescribed central nervous system depressant drugs with sedative, muscle relaxant, and anticonvulsant activities. Chronic use does result in moderate dependence and tolerance to the drug. The use of alcohol in conjunction with the benzodiazepines has been shown to have a greater suppressive effect to the central nervous system than that attributable to either chemical alone. Benzodiazepines are usually administered orally and are absorbed rapidly. The metabolism of benzodiazepines is mainly in the liver and excreted in the urine as a variety of structurally related metabolites. Metabolic similarities include removal of substituents from the B ring of the 1,4-benzodiazepines and alpha hydroxylation of the triazolobenzodiazepines, hydroxylation of the 3 position carbon of the B ring, and conjugation of hydroxylated metabolites followed by urinary excretion as glucuronides.[6]

The Benzodiazepines 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 Benzodiazepine 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 2 ng/ml.

The Benzodiazepines Direct ELISA Kit is a sensitive *in vitro* test to detect the presence of Benzodiazepines in samples such as whole blood, serum, plasma, and urine. It avoids extraction of urine or blood sample for measurement. It employs an Oxazepam 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 results in extremely small sample size reducing matrix effects and interference with binding protein(s) or other macromolecules.

Components

Materials Provided	96 Tests
Microwells coated polyclonal anti-Oxazepam	12 x 8 x 1
Benzo-Conjugate	12 mL
Immunalysis Positive Reference Standard	2 mL
Negative Standard	1 mL
Stop Solution	12 mL
TMB Substrate	11 mL

Reagents and Equipment Required but Not Provided.

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

Storage/Stability

Store the kit at 2–8 °C. The expiration date of the kit is stated on the label. Keep microwells sealed in a dry bag with desiccants. Do not expose test reagents to heat, sun or strong light.

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.

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Dilute forensic specimens, to the necessary range with phosphate buffered saline, pH 7.0. (Urine samples are normally diluted 1:10 for Oxazepam 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 (20–25 °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

If the average sample absorbance is equal to or less than the average absorbance of the laboratory positive reference standard the sample is POSITIVE for benzodiazepines.

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

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.

Example of a Standard data

The following table represent a typical dose/response Data.

Oxazepam (ng/mL)	Absorbance
0	3.043
25	0.750
50	0.548
100	0.388

The dose/response data 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

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