# CEDIA® Barbiturate Assay

Thermo SCIENTIFIC

**IVD** **For In Vitro Diagnostic Use**

**REF** 10017365 (3 x 17 mL Indiko Kit)
100084 (3 x 17 mL Kit)
100093 (65 mL Kit)
1661213 (495 mL Kit)

## Intended Use
The CEDIA® Barbiturate Assay is an in-vitro diagnostic medical device intended for the qualitative and semi-quantitative assay of barbiturates in human urine.

***The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/ mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgement should be applied to any drug of abuse test result particularly when preliminary positive results are used.***

## Summary and Explanation of The Test
Barbiturates belong to a broad classification of CNS-depressant drugs known as sedative/ hypnotics.[2-4] When used as a substance of abuse, barbiturates are usually taken orally in pill form, but habitual users and addicts have been known to dissolve the compounds and inject them hypodermically.[2,3,5]

Depending on the degree of lipid solubility, barbiturates are commonly characterized as short, intermediate, or long acting.[2,6] Half lives range from 20 to 120 hours.[4,6] Barbiturates are variously metabolized by the liver, some being excreted in the urine mainly as active and inactive metabolites and others mainly as unchanged drug.[4,6] Depending on the specific barbiturate taken, urine may test positive for approximately 30 hours after administration or as long as several weeks.[4]

The CEDIA Barbiturate Assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[7] This assay is based on the bacterial enzyme ß-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form fully active enzyme that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, drug in the sample competes with drug conjugated to one inactive fragment of β-galactosidase for antibody binding site. If drug is present in the sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme. If drug is not present in the sample, antibody binds to drug conjugated on the inactive fragment, inhibiting the reassociation of inactive enzyme fragments, and no active enzyme will be formed. The amount of active enzyme formed and resultant absorbance change are proportional to the amount of drug present in the sample.

## Reagents
1 **EA Reconstitution Buffer:** Contains piperazine-N,N-bis [2-ethanesulfonic acid], 2.2 µg/mL monoclonal antibodies to barbiturates, buffer salts, stabilizer, and preservative.
1a **EA Reagent:** Contains 0.171 g/L enzyme acceptor, buffer salts, detergent and preservative.
2 **ED Reconstitution Buffer:** Contains piperazine-N,N-bis [2-ethanesulfonic acid]; buffer salts and preservative.
2a **ED Reagent:** Contains 17.1 µg/L enzyme donor conjugated to a barbiturate derivative, 1.67 g/L chlorophenol red-ß-D-galactopyranoside, stabilizer and preservative.

***Additional Materials:***
Alternative Bar Code Labels (Cat. Nos. 100084 and 100093 only. Refer to analyzer specific application sheet for directions on usage.) Empty analyzer bottles for EA/ED solution pour-over (Cat. No. 100093). Empty analyzer bottle for ED solution pour-over (Cat. No. 1661213 only.)

***Additional Materials Required (sold seperately):***
CEDIA Negative Calibrator
CEDIA Multi-Drug Calibrator, Primary Cutoffs or Primary Clinical Cutoffs, 300 ng/mL
CEDIA Multi-Drug Calibrator, Secondary Cutoffs or Optional Cutoffs, 200 ng/mL
CEDIA Multi-Drug Intermediate Calibrator
CEDIA Multi-Drug High Calibrator
For 300 ng/mL Cutoff: Multi-Drug Control Set or Multi-Drug Clinical Control Set
For 200 ng/mL Cutoff: Specialty Control Set or Multi-Drug Optional Control Set

## ⚠ Precautions and Warnings
The reagents contain sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

## Reagent Preparation and Storage
See below for preparation of the solutions for Hitachi analyzers. For all other analyzers, refer to the analyzer specific application sheet. Remove the kit from refrigerated storage immediately prior to preparation of the solutions.

Prepare the solutions in the following order to minimize possible contamination:

***R2 Enzyme donor solution:*** Connect Bottle 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at room temperature (15-25°C). Mix again. Record the reconstitution date on the bottle label.

***R1 Enzyme acceptor solution:*** Connect Bottle 1a (EA Reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 5 minutes at room temperature (15-25°C). Mix again. Record the reconstitution date on the bottle label.

**Cat. No.100093 - Hitachi 717, 911, 912 or 914 analyzer :** Transfer the reconstituted reagents into the corresponding empty R1 and R2 100 mL bottles supplied with kit. ***Hitachi 917/ Modular analytics P system:*** Use the reconstituted reagents without transfer of bottles. Discard the empty 100 mL bottles.

**Cat. No. 1661213 - Hitachi 747 analyzer/ Modular analytics D system:** Use the funnel provided to transfer a portion of the R2 Solution into the appropriately labeled empty R2 Solution bottle provided.

***NOTE 1:*** The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.
***NOTE 2:*** Avoid cross-contamination of reagents by matching reagent stoppers to the proper reagent bottle. The R2 Solution should be yellow-orange in color. A dark red or purple-red color indicates that the reagent has been contaminated and must be discarded.
***NOTE 3:*** The R1 and R2 Solutions must be at the reagent compartment storage temperature of the analyzer before performing the assay. Refer to the analyzer specific application sheet for additional information.
***NOTE 4:*** To ensure reconstituted EA solution stability, protect from prolonged continuous exposure to bright light.

Store reagents at 2-8°C. **DO NOT FREEZE.** For stability of the unopened components, refer to the box or bottle labels for the expiration date.

**R1 Solution:** 60 days refrigerated on analyzer or at 2-8°C.
**R2 Solution:** 60 days refrigerated on analyzer or at 2-8°C.

## Specimen Collection and Handling
Collect urine samples in clean glass or plastic containers. Centrifuge specimens with high turbidity before testing. Treat human urine as potentially infectious material. Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine samples can affect the test results.

*The Mandatory Guidelines for Federal Workplace Drug Testing Programs; Final Guidelines; Notice* recommend that specimens that do not receive an initial test within 7 days of arrival at the laboratory should be placed into secure refrigeration units.[8]

## Assay Procedure
Chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates and timing the reaction accurately can be used to perform this assay. Application sheets with specific instrument parameters are available from Microgenics, a part of Thermo Fisher Scientific.

Additional barcode labels are provided for semi-quantitative determination with the 17 mL and 65 mL kits only. To use, over label each bottle with the correct label.

## Quality Control and Calibration[9]
***Qualitative analysis***
For qualitative analysis of samples, use the Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optional Cutoffs (depending on the selected cutoff), to analyze results. See the analyzer specific application sheet.

***Semi-quantitative analysis***
For semi-quantitative analysis of samples, use the CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optional Cutoffs in conjunction with the CEDIA Negative Calibrator, and the Multi-Drug Intermediate and High Calibrators to analyze results. See the analyzer specific application sheet.

Recalibrate the test if reagents are changed or if control results are outside of established limits. Each laboratory should establish its own control frequency. Good laboratory practice suggests that controls be run each day patient samples are tested and each time calibration is performed. It is recommended that two levels of controls be run; one 25% above the selected cutoff and the other 25% below the selected cutoff. Use the CEDIA Multi- Drug Control Set or Multi-Drug Clinical Control Set, (300 cutoff) or Speciality Control Set or Multi-Drug Optional Control Set, (200 cutoff) for quality control. Recalibrate the test if reagents are changed or if control results are outside of established limits. Each laboratory should establish its own control frequency. Values obtained for the controls should fall within specified limits. If any trends or sudden shifts in values are detected, review all operating parameters or contact Thermo Fisher Scientific Customer Technical Support for further assistance. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

## Results and Expected Values

***Qualitative results***
The CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optional Cutoffs, (depending on selected cutoffs), is used as a reference in distinguishing between positive and negative samples. Samples producing a response value equal to or greater than the response value of the calibrator are considered positive. Samples producing a response value less than the response value of the calibrator are considered negative. Refer to analyzer specific application sheet for additional information.

***Semi-quantitative results***
The CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optinal Cutoffs, used in conjunction with the Negative and the Multi-Drug Intermediate and High Calibrators, can be used to estimate relative concentration of barbiturates. Refer to the analyzer specific application sheet for detailed information.

Care should be taken when reporting concentration results since there are many other factors that may influence a urine test result such as fluid intake and other biological factors.

## Limitations

1. A positive test result indicates the presence of barbiturate; it does not indicate or measure intoxication.
2. Other substances and/or factors not listed may interfere with the test and cause false results (eg., technical or procedural errors)

## Specific Performance Characteristics

Typical performance data results obtained on the Hitachi 717 analyzer are shown below.[10] The results obtained in your laboratory may differ from these data.

***Precision***
Measured precision studies, using packaged reagents and calibrators, yielded the following results in mA/min with a Hitachi 717 analyzer following NCCLS modified replication experiment guidelines.

| Within-run Precision | | | | |
|---|---|---|---|---|
| **ng/mL** | 200 | 225 | 300 | 375 |
| **n** | 120 | 120 | 120 | 120 |
| **$\bar{x}$** | 353.6 | 368.0 | 403.1 | 435.4 |
| **SD** | 5.4 | 4.1 | 3.5 | 4.9 |
| **CV** | 1.5% | 1.1% | 0.9% | 1.1% |

| Total Precision | | | | |
|---|---|---|---|---|
| **ng/mL** | 200 | 225 | 300 | 375 |
| **n** | 120 | 120 | 120 | 120 |
| **$\bar{x}$** | 353.6 | 368.0 | 403.1 | 435.4 |
| **SD** | 10.6 | 10.8 | 10.8 | 12.0 |
| **CV** | 3.0% | 2.9% | 2.7% | 2.8% |

***Accuracy***
Six hundred and nine samples were assayed with CEDIA Barbiturate assay on the Hitachi 717 using a commerical EIA method for barbiturates as reference. Results were as follows:

**A. 200 ng/mL Cutoff**

| EIA \ CEDIA | + | - |
|---|---|---|
| + | 111 | 0 |
| - | 1* | 497 |

**B. 300 ng/mL Cutoff**

| EIA \ CEDIA | + | - |
|---|---|---|
| + | 103 | 0 |
| - | 5† | 501 |

* The samples were tested by GC/MS and were found to contain 152 ng/mL phenobarbital.
† The samples were tested by GC/MS and were found to contain 471-1578 ng/mL phenobarbital.

***Specificity***
The following parent compounds and metabolites, when tested with the CEDIA Barbiturate assay, 200 ng/mL cutoff protocol, yielded the following percent cross-reactivity results:

| Compound | Concentration Tested (ng/mL) | % Cross-Reactivity |
|---|---|---|
| Secobarbital | 200 | 100 |
| Amobarbital | 207 | 109 |
| Aprobarbital | 195 | 80 |
| Barbital | 1,000 | 18 |
| Butabarbital | 198 | 78 |
| Butalbital | 213 | 92 |
| Cyclopentobarbital | 190 | 115 |
| Pentobarbital | 270 | 66 |
| Phenobarbital | 195 | 83 |
| Talbutal | 130 | 160 |

Structurally unrelated compounds were tested with CEDIA Barbiturate assay, 200 ng/mL cutoff protocol, and gave a negative response when tested at the concentrations listed below.

| Compound | ng/mL | Compound | ng/mL |
|---|---|---|---|
| Acetaminophen | 500,000 | Ibuprofen | 500,000 |
| Acetylsalicylic acid | 500,000 | Levothyroxine | 50,000 |
| Amoxicillin | 100,000 | Methadone | 500,000 |
| Amphetamine | 500,000 | Methamphetamine | 500,000 |
| Benzoylecgonine | 500,000 | Nifedipine | 500,000 |
| Captopril | 500,000 | Phencyclidine | 500,000 |
| Chlordiazepoxide | 100,000 | Propoxyphene | 500,000 |
| Cimetidine | 500,000 | Ranitidine | 500,000 |
| Diazepam | 500,000 | Salicyluric acid | 500,000 |
| Digoxin | 100,000 | 11-nor-$\Delta^9$-THC-COOH | 10,000 |
| Enalapril | 500,000 | Verapamil | 500,000 |
| Fluoxetine | 500,000 | | |

No interference was observed from the following substances added to the normal endogenous concentrations found in urine when tested with the CEDIA Barbiturate assay:

| Substance | Concentration | Substance | Concentration |
|---|---|---|---|
| Acetone | ≤ 1.0 g/dL | Hemoglobin | ≤ 0.3 g/dL |
| Ascorbic acid | ≤ 1.5 g/dL | Human serum albumin | ≤ 0.5 g/dL |
| Creatinine | ≤ 0.5 g/dL | Oxalic acid | ≤ 0.1 g/dL |
| Ethanol | ≤ 1.0 g/dL | Riboflavin | ≤ 7.5 mg/dL |
| Galactose | ≤ 10 mg/dL | Sodium Chloride | ≤ 6.0 g/dL |
| γ-globulin | ≤ 0.5 g/dL | Urea | ≤ 2.0 g/dL |
| Glucose | ≤ 1.5 g/dL | | |

***Sensitivity***
For the Qualitative application, the limit of detection (LOD), was 13.7 ng/mL and 15.2 ng/mL for the 200 ng/mL and 300 ng/mL cutoff protocols, respectively.

For the Semi-quantitative application, the LOD, was 24.2 ng/mL and 36.3 ng/mL for the 200 ng/mL and 300 ng/mL cutoff protocols, respectively.

**References**


1. Hawks RL. Analytical methodology. In: Hawks RL, Chiang CN, eds. Urine Testing for Drugs of Abuse. NIDA Research Monograph 1986; 73: 30-41.
2. Miller NS, Gold MS. Sedative/hypnotics. In: Giannini AJ, Slaby AE, eds. Drugs of Abuse. Oradell, NJ: Medical Economics Books, 1989.
3. Jones KL, Shainberg LW, Byer CO. Drugs and Alcohol. 3rd ed. New York, NY: Harper & Row; 1979.
4. Julien RM. A Primer of Drug Action. 6th ed. New York, NY: WH Freeman & Co; 1992.
5. Miller NS, Gold MS. Sedative-hypnotics: Pharmacology and use. J Fam Practice. 1989; 29: 665-670.
6. Baselt RC, Cravey RH. Disposition of Toxic Drugs and Chemicals In Man. 4th ed. Foster City, Calif.: Chemical Toxicology Institute; 1995
7. Henderson DR, Friedman SB, Harris JD, et al. CEDIA™, a new homogeneous immunoassay system. Clin Chem 1986; 32: 1637-1641.
8. Notice of Mandatory Guidelines for Federal Workplace Drug Testing Program: Final guidelines. Federal Register. 1994; 110 (June 9): 11983. (Revised Guidelines expected in 2002).
9. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
10. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.



Microgenics Corporation
46500 Kato Road
Fremont, CA 94538 USA
US Customer and
Technical Support:
1-800-232-3342

EC REP
Microgenics GmbH
Spitalhofstrasse 94
D-94032 Passau, Germany
Tel: +49 (0) 851 886 89 0
Fax: +49 (0) 851 886 89 10


CE

For insert updates go to:
www.thermoscientific.com/diagnostics

**Other countries:**
Please contact your local Thermo Fisher Scientific representative.




10006441-5-EN
2014 12


Thermo SCIENTIFIC