CEDIA™ Phenobarbital II Assay

thermo scientific

IVD

For In Vitro Diagnostic Use

REF 100003 (17 mL, 17mL Kit)

Rx Only

Read Highlighted Changes: Revised October 2022

Intended Use
The CEDIA™ Phenobarbital II Assay is an in vitro medical device intended for the quantitation of phenobarbital in human serum or plasma.

Summary and Explanation of the Test
Phenobarbital has been widely prescribed for the treatment of epilepsy, particularly for controlling focal motor or sensory and grand mal seizures.[1,2] After oral doses of 2 to 3 mg/kg, phenobarbital is almost completely absorbed with peak levels achieved by 12 to 18 hours.[3] Phenobarbital in circulation is approximately 40% to 50% bound to plasma proteins with relatively low association constants.[4] The major metabolic pathway of phenobarbital is hydroxylation of the phenyl ring to p-hydroxyphenobarbital, an agent devoid of hypnotic activity, which is then excreted in the urine in equal amounts of the free form and the form conjugated with glucuronic acid.[4] Phenobarbital concentrations of 15-40 µg/mL in serum are normally considered to be within the therapeutic range for maximum seizure control.[5]

The need for monitoring phenobarbital concentrations is due to the narrow therapeutic index and the wide variability in individual rates of drug absorption, metabolism, and clearance.[7] Toxicity of phenobarbital therapy includes sedation, nystagmus, ataxia, paradoxical excitement, blood dyscrasia (including coagulation defects in neonates of mothers given the drug during pregnancy), non-specific hepatic changes, rash (including severe exfoliative forms), osteomalacia, the shoulder-hand syndrome and coma.[3,8,9,10,11] In combination with other clinical information, monitoring serum or plasma phenobarbital levels will provide physicians with an essential tool to aid in adjusting dosage and achieving optimal therapeutic effect, while avoiding both subtherapeutic and harmful toxic drug levels.

The CEDIA Phenobarbital II Assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[12]

The assay is based on the bacterial enzyme β-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, the displacement format is used to provide improved sensitivity and precision through reduced background noise and concomitant increase of signal-to-noise ratio. In the assay, phenobarbital in the sample displaces a fraction of the antibody ED-phenobarbital conjugate complex. Subsequently, EA reagent is added and the reactants are incubated to allow complementation with free ED-phenobarbital conjugate.

The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of analyte present in the sample.[12]

Reagents
- **1 Enzyme Donor Reconstitution Buffer:** Contains MOPS (3-[N-morpholino]-propanesulfonic acid), buffer salts, and preservative.
- **1a Enzyme Donor Reagent:** Contains 38 µg/L Enzyme donor conjugated to phenobarbital, 16.1 mg/L mouse monoclonal anti-phenobarbital antibody, 1.64 g/L chlorophenol red-β-D-galactopyranoside, buffer salts, and preservative.
- **2 Enzyme Acceptor Reconstitution Buffer:** Contains MOPS (3-[-morpholino]-propanesulfonic acid buffer), buffer salts, 0.945g goat anti-mouse antibodies, stabilizers and preservative.
- **2a Enzyme Acceptor Reagent:** Contains 0.171 g/L Enzyme acceptor, releasing agent, buffer salts, and preservative.

Additional Materials Required (but not provided):

REF	Kit Description
100007	CEDIA Core TDM Multi-Cal

Commercial Control(s)- Consult Customer Technical Support for recommendations on suitable control material.

⚠ Precautions and Warnings
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DANGER: Powder reagent contains ≤56% w/w bovine serum albumin (BSA), ≤2% w/w Sodium azide and ≤0.5% drug-specific antibody (mouse). Liquid reagent contains ≤1.0% Bovine serum, ≤0.3% Sodium azide and ≤0.1% goat anti-mouse antibody.
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
EUH032 - Contact with acids liberates very toxic gas.

Avoid breathing dust/mist/vapors/spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

This product contains chemical(s) known to the State of California to cause cancer and/or birth defects or other reproductive harm.

Reagent Preparation and Storage
Consult application sheet for any exceptions from the following instructions. Remove the kit from refrigerated storage (2-8°C) immediately prior to preparation of the solutions.

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

R 1 Enzyme donor solution : Connect Bottle 1a (ED Reagent) to Bottle 1 (ED 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 15-25°C. Mix again. Record the reconstitution date on the bottle label.
R 2 Enzyme acceptor solution : Connect Bottle 2a (EA Reagent) to Bottle 2 (EA 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 15-25°C. Mix again. Record the reconstitution date on the bottle label.

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 R1 Solution should be yellow-orange in color. A 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
Serum or plasma (Na or Li heparin; Na EDTA) samples are suitable for use in the CEDIA Phenobarbital II assay. Care should be taken to preserve the chemical integrity of the serum or plasma sample from the time it is collected until the time it is assayed. Cap samples, store at 2-8°C and assay within 24 hours after collection. If the assay cannot be performed within 24 hours, or if the sample is to be shipped, cap the sample, and keep it frozen. Store samples at -20°C and assay within 2 weeks. To protect the integrity of the sample do not induce foaming and avoid repeated freezing and thawing. Centrifuge specimens containing particulate matter.

Some therapeutic drug concentrations are reduced when sample is stored in separator tube for a prolonged period of time (> 2 hours).[13]

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. Two (2) point calibration is used with this assay. In the case of Hitachi analyzers, use CEDIA Core TDM Multi-Cal Low Calibrator as S1 and CEDIA Core TDM Multi-Cal High Calibrator as S2. For exact analyzer application parameter settings, refer to each instrument specific application sheet, which is available from Microgenics Customer Technical Support.

Hitachi 911 analyzer: If the barcode is not read by the analyzer, the numerical sequence on the bar code label can be entered manually via the keyboard.

Quality Control and Calibration
Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters. Contact Customer Technical Support for further assistance and recommendations on suitable control material. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements. **NOTE:** Reassess control targets and ranges following a change of reagent lot.

Calibration Frequency - Two point calibration is recommended:

- after reagent bottle change
- after reagent lot change
- as required following quality control procedures

Results and Expected Values

In most patients, a therapeutic response is achieved with phenobarbital concentrations in the 15-40 µg/mL (65-172 µmol/L) range.[5] Some patients may require phenobarbital concentrations outside this range to obtain effective seizure control. Therefore, therapeutic range is provided only as a guide and individual patient results should be interpreted in conjunction with other clinical symptoms and individual clinical history.

The CEDIA Phenobarbital II Assay is designed to quantitate patient sample phenobarbital concentrations between 1.8 µg/mL and 80 µg/mL (CEDIA TDM Multi-Cal High Calibrator). Specimen results outside this range should be reported as either > 80 µg/mL or < 1.8 µg/mL (assay sensitivity limit).

Specimens quantitating greater than 80 µg/mL can also be diluted one part sample with one part Multi-Cal Low Calibrator and reassayed. The value obtained on reassay should be derived as:

Actual Value = (2 x diluted value) – Multi-Cal Low Calibrator value

Use the following conversion factor to convert µg/mL to µmol/L:

µg/mL x 4.31 = µmol/L
µmol/L x 0.232 = µg/mL

Limitations

1. The CEDIA Phenobarbital II Assay performance has not been established with body fluids other than human serum and plasma (Na or Li heparin; Na EDTA).
2. The incidence of patients having antibodies to E. coli β-galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high results that do not fit the clinical profile. If this occurs, contact Customer Technical Support for assistance.
3. This assay was validated on analyzers utilizing an integral cell wash. If your analyzer does not have an integral cell wash, contact your local Thermo Fisher Scientific representative for an alternative procedure.

Specific Performance Characteristics

Typical performance data obtained on the Hitachi 911 analyzer are shown below.[13] The results obtained in your laboratory may differ from these data.

Precision

Measured precision studies using packaged reagents, pooled human serum and control sera yielded the following results in µg/mL with a Hitachi 911 analyzer following NCCLS modified replication experiment guidelines:

	Within-run precision			Total precision		
N	120	120	120	120	120	120
$\bar{x}$ (µg/mL)	9.4	25.9	52.7	9.4	25.9	52.7
SD (µg/mL)	0.2	0.5	0.9	0.5	1.0	1.9
CV%	2.1	1.9	1.7	5.3	3.8	3.6

Method Comparison

A comparison using the CEDIA Phenobarbital II assay (y) with a commercial fluorescence polarization immunoassay (x) gave the following correlation (µg/mL):

Linear regression

y = 1.0828x - 0.74
r = 0.9938

Number of samples measured: 89
The sample concentrations were between 1.7 and 73.3 µg/mL.

Linearity

To assess the linearity of the assay, a high sample was diluted with the Core TDM Multi-Cal Low Calibrator. The percent recovery was then determined by dividing the assayed value by the expected value.

% High Sample	Expected Value (µg/mL)	Assayed Value (µg/mL)	% Recovery
100.0	-	84.6	-
90.0	78.4	75.6	96.4
80.0	69.8	66.0	94.6
70.0	61.1	60.4	98.8
60.0	52.5	52.9	101.9
50.0	43.8	44.6	101.9
40.0	35.2	34.7	98.8
30.0	26.5	26.6	100.4
20.0	17.8	14.7	99.1
10.0	9.2	9.1	99.1
0.0	-	0.6	-

Recovery

To assess the recovery of the assay, phenobarbital was added to a low phenobarbital sample and then diluted with an analyte-free sample. The percent recovery was then determined by dividing the assayed value by the expected value.

% High Sample	Expected Value (µg/mL)	Assayed Value (µg/mL)	% Recovery
100.0	-	85.4	-
90.0	83.0	78.1	94.0
80.0	73.8	71.6	97.1
70.0	64.5	63.5	98.6
60.0	55.3	55.8	100.8
50.0	46.0	46.7	101.4
40.0	36.8	37.0	100.6
30.0	27.6	27.7	100.6
20.0	18.3	17.9	97.7
10.0	9.1	8.9	98.5
0.0	-	0.1	-

Specificity

The following compounds have been tested for cross-reactivity in the CEDIA Phenobarbital II Assay.

Compound	Concentration tested (µg/mL)	% Cross-reactivity
1,3-dimethylbarbituric acid	1000	< 0.12
2-Phenyl-2-ethylmalonamide	1000	< 0.12
5-(p-Hydroxyphenyl)-5-phenylhydantoin	1000	< 0.12
Amitriptyline	1000	< 0.12
Aprobarbital	1000	5.3
Barbital	2000	1.6
Butabarbital	1000	2.3
Carbamazepine -10, 11-epoxide	1000	< 0.12
Carbamazepine	1000	< 0.12
Chlorazepate	2000	< 0.12
Chlorpromazine	1000	< 0.12
Diazepam	1000	< 0.12
Ethotoin	1000	< 0.12
Ethosuximide	1000	< 0.12
Glutethimide	1000	< 0.12
Imipramine	2000	< 0.12
Mephenytoin	1000	< 0.12
Methsuximide	1000	< 0.12
Pentobarbital	1000	< 0.12
Phenytoin	400	0.9
p-Hydroxyphenobarbital	2000	< 0.12
Primidone	1000	0.5
Promethazine	1000	< 0.12
Secobarbital	2000	2.2
Sulthiame	1000	< 0.12
Valproic Acid	2000	0.7

No interference was found in CEDIA Phenobarbital II Assay with:

Substance	Concentration	Substance	Concentration
Bilirubin	≤ 66 mg/dL	Total protein	≤ 13 g/dL
Hemoglobin	≤ 1000 mg/dL	Triglyceride	≤ 1000 mg/dL
Rheumatoid factor	≤ 180 IU/mL		

Amobarbital (> 20%) and Mephobarbital (> 100%) show significant interference with the CEDIA Phenobarbital II Assay.

Sensitivity
The minimum detectable concentration of the CEDIA Phenobarbital II Assay is 1.8 µg/mL (7.76 µmol/L). This value was determined by calculating the concentration of phenobarbital which would give a response equal to two standard deviations above that of the Core TDM Multi-Cal Low Calibrator.

Reference

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13. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

Glossary:
http://www.thermofisher.com/symbols-glossary

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