This package insert follows the Pharmaceuticals, Medical devices and Other Therapeutic Products Act of Japan.

In Vitro Diagnostics
Marketing Notification No. 13A2X00197218104

**Revised: January 2020 (6th edition)
*Revised: January 2017 (5th edition)

This package insert must be read carefully prior to use.

Phenobarbital assay kit
(Classification No.: 30401000)

Nanopia TDM Phenobarbital

General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
4. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
5. Carefully read the operating instructions for each type of automated analyzers prior to using this product. Parameters for each type of analyzers are available, and can be requested from SEKISUI MEDICAL CO., LTD. if required.
6. Perform a quality control test prior to assay to ensure accuracy.
7. PB Antibody Solution 1 contains human-derived components that have been shown to be negative for HBs antigens, HIV antibodies (AIDS virus antibodies), and HCV antibodies. However, these reagents (as well as the samples) should be considered potentially infectious and handled with great care.

Description (Kit Components)

Component: Ingredients
PB Antibody Solution 1:
Anti-phenobarbital mouse monoclonal antibody
PB Latex Reagent 2:
Phenobarbital-coated latex

Intended Use **

Measurement of phenobarbital in serum or plasma

Since Hauptmann introduced phenobarbital into clinical practice in 1912, this drug has been widely used as an anticonvulsant for the treatment of epilepsy, especially focal seizures, sensory seizures, and grand mal epilepsy. The maximum blood level of phenobarbital is attained at 12−18 hours after oral administration at a dose of 2−3 mg/kg. The protein-binding rate of phenobarbital is relatively low, and approximately 40−50% of this drug is bound to plasma protein in the bloodstream. Free phenobarbital not bound to plasma protein exhibits a pharmacological effect. Phenobarbital is mostly absorbed from the stomach and partially absorbed from the intestine, and is metabolized in the liver. With is hydroxylation of the benzene ring, it is metabolized to p-hydroxyphenobarbital and then excreted in the urine without further metabolism or as a glucuronic acid conjugate.[1)]

Toxicities of phenobarbital can be dose-dependent or dose-independent. Most of the dose-dependent symptoms of toxicity are neurological symptoms, such as sedation, nystagmus, ataxia, and coma. Reflex excitation; coagulopathy; abnormal hepatic function; skin rash; osteomalacia; and neck, shoulder and arm syndrome are symptoms of toxicity that are considered to be dose-independent. Farewell et al. recently reported that intellectual disability is observed in children on long-term phenobarbital therapy.[2)]

Monitoring the blood level of phenobarbital is useful for adjusting the dosage so that the optimum clinical response can be obtained without symptoms of toxicity.[3),4)]

Assay Principle

1. Assay Principle

When a certain amount of anti-phenobarbital antibody is added and reacted with a sample, consumption of the antibody depends on its content in the sample. When phenobarbital-coated latex is added, residual anti-phenobarbital antibody reacts with the latex and forms aggregates.

Since the extent of aggregation depends on the phenobarbital concentration in the sample, the phenobarbital concentration can be determined by measuring aggregation as the change of absorbance.

Sample (phenobarbital) + Anti-phenobarbital antibody ⟶ Antigen-antibody reaction

Unreacted anti-phenobarbital antibody + Phenobarbital-coated latex
⟶ Aggregation by antigen-antibody reaction

2. Features

1) Because a highly specific monoclonal antibody is used, this product shows excellent sensitivity and accuracy.
2) Liquid reagents, ready-to-use.
3) Applicable to various automated analyzers.

Procedural Precautions *

1. Properties of Samples and Sampling Methods

1) Samples
Serum and plasma (heparin plasma, EDTA plasma and citrated plasma) may be used.
2) Storage of samples
If serum or plasma samples cannot be measured on the day of separation, store them as follows. Avoid repetition of freezing and thawing.
2–8°C: for tests within 7 days
≤ -20°C: for tests within 3 months
Bring samples to room temperature (15–30 °C) before use.
3) Caution must be exercised, because a separating agent, etc. in the blood collection tube may affect assay values.[5)]

4) Sampling should be performed after removing insoluble matter from the sample.

2. Interfering substances

1) Assay results are not affected by free bilirubin (up to 20 mg/dL), conjugated bilirubin (up to 20 mg/dL), hemoglobin (up to 500 mg/dL), ascorbic acid (up to 50 mg/dL), formazine turbidity (up to 2500 FTU), or rheumatoid factors (up to 450 U/mL).
2) Because mouse antibody is used in the assay, artifactual elevation of results may occur if the sample contains human anti-mouse antibody. In this case, perform re-measurement by another method.
3) Cross-reactivity
The following table summarizes drugs related to phenobarbital and cross-reactivity between phenobarbital and other drugs.

Substance	Concentration tested (μg/mL)	Cross-reactivity (%)
1,3-Dimethylbarbituric acid	1000	0.19
2-Phenyl-2-ethylmalonamide	1000	0.24
5-(p-Hydroxyphenyl)-5-phenylhydantoin	1000	0.17
Amitriptyline	1000	0.09
Aprobarbital	1000	0.11
Barbital	2000	0.04
Butabarbital	1000	0.21
Carbamazepine	1000	0.28
Carbamazepine-10,11-epoxide	1000	0.26
Clorazepate	2000	0.08
Chlorpromazine	2500	0.10
Diazepam	1000	0.13
Ethosuximide	1000	0.16
Ethotoin	1000	0.17
Glutethimide	1000	0.14
Imipramine	4000	0.07
Mephenytoin	3000	0.10
Methsucimide	5000	0.05
Phenytoin	400	0.55
p-Hydroxyphenobarbital	2000	0.02
Primidone	1000	0.33
Promethazine	1500	0.12
Secobarbital	2000	0.15
Valproic acid	7000	0.00

3. Others

1) Always use TDM Calibrator for Nanopia for calibration.
2) Precautions for assay range
If the concentration of a target substance in the sample exceeds the measurement range, dilute the sample with a separately sold diluent (manufactured by SEKISUI MEDICAL CO., LTD.), and perform re-measurement.

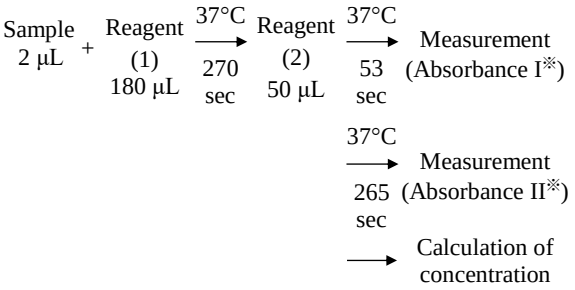
Dosage/Administration (Assay Procedure) ＊

1. Preparation of reagents

Reagent (1): PB Antibody Solution 1 is ready to use.
Reagent (2): PB Latex Reagent 2 is ready to use. Before using this product, gently invert the PB Latex Reagent 2 bottle to mix it thoroughly, and check that there are no bubbles.

2. Assay Procedure

This product is compatible with various types of automated analyzer. An example of the assay procedure is indicated below.



※Absorbance I and II: Absorbance at 700 nm
Calibration material: TDM Calibrator for Nanopia (manufacture's assigned value)

Assessment of Assay Results ＊＊

1. Reference standard range

The effective concentration of phenobarbital has been reported to be 10−40 μg/mL.[6] However, there is individual variation of drug metabolism, so higher blood levels beyond this range may be required in some patients. Therefore, interpretation of assay results should also be based on the patient's clinical findings and other examination results.
It is recommended that the blood concentration of phenobarbital should be measured in a sufficient number of samples for statistical analysis and that its clinical effective concentration should be determined by each medical institution.

2. There may be reactions or interfering reactions with non-target substances. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

Performance

1. Sensitivity

1) The change of absorbance per minute with the standard solution (0 μg/mL) is 0.110−0.200.
2) The ratio between the change of absorbance per minute with the standard solution (0 μg/mL) and that with another standard solution (5 μg/mL) is 60−80%.

2. Accuracy: 80–120 % of the expected assay value

3. Within-run Reproducibility:
Coefficient of variation ≤ 10 %
(Test methods used for 1.–3. are in-house methods.)

4. Measurement Range[7]: (On Hitachi 7170S automated analyzer)
0.8–80 μg/mL

5. Correlation[7]

1) Serum N=60 r=0.992 y=0.95x+0.21
Control method: Approved in vitro diagnostic (enzyme immunoassay)
2) Plasma N=103 r=0.992 y=0.96x+0.88
Control method: Approved in vitro diagnostic

(enzyme immunoassay)

6. Standard Material

Phenobarbital (U.S. Pharmacopoeia)

Precautions for Use or Handling *

1. Precautions for Handling (to Ensure Safety)

1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.
2) Sodium azide is added as an antiseptic agent in the PB Antibody Solution 1 and PB Latex Reagent 2. Therefore, if the reagent comes in accidentally contact with eyes, mouth or skin, rinse immediately with ample water as first aid, and consult the doctor if required.

2. Precautions for use

1) This product should be stored as directed, without freezing. Freezing can deteriorate the reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.
2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
3) Do not replenish the reagents.
4) Do not mix materials from different kit lot numbers.
5) Do not perform the assay under direct sunlight

3. Precautions for Disposal

1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121ºC for 20 minutes.
2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.
5) Sodium azide has been added as an antiseptic agent in the PB Antibody Solution 1 and PB Latex Reagent 2. It can react with lead or copper pipes to produce the highly explosive metal azide. Therefore, the reagent should be flushed with large amounts of water during disposal.

4. Other precautions

Do not use the containers for other purposes.

Storage and Shelf Life *

1. Storage temperature: 2–8°C

2. Shelf life: 2 years from the date of manufacture
(The expiration date is printed on the outer package.)

Packaging

Name		Package
Nanopia TDM Phenobarbital	PB Antibody Solution 1	1 × 36 mL
	PB Latex Reagent 2	1 × 10 mL

Constituent reagents are available in other configurations. For further details please contact SEKISUI MEDICAL CO., LTD.

References * *

1) Buchthal F., Svensmark O.: Serum concentrations of diphenylhydantoin (phenytoin) and phenobarbital and their relation to therapeutic and toxic effects, Psychiat. Neurol. Neurochir., 74 : 117−135, 1971.
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3) Kutt He., Penry K.: Usefulness of blood levels of antiepileptic drugs, Arch. Neurol., 3 : 283−288, 1974.
4) Waddell W. J., Butler T. C.: The distribution and excretion of phenobarbital, J. Clin. Invest., 36 : 1217−1226, 1957.
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7) In house data, SEKISUI MEDICAL CO., LTD.

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