In Vitro Diagnostics
Marketing Notification No. 13A2X00197218105
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*Revised: July 2019 (7th edition)

This package insert must be read carefully prior to use.

Phenytoin assay kit
(Classification No.: 30402000)

Nanopia TDM Phenytoin

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. PHT 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
PHT Antibody Solution 1:
Anti-phenytoin mouse monoclonal antibody
PHT Latex Reagent 2:
Phenytoin-coated latex

Intended Use

Measurement of phenytoin in serum or plasma

Phenytoin (diphenylhydantoin) is one of the drugs used to treat epilepsy. It is most widely prescribed as an anticonvulsant for grand mal seizures (mostly kinetic), focal seizures, and temporal lobe epilepsy.[1)] Phenytoin is usually administered orally. It is almost completely absorbed (85−95%) from the small intestine.[2)] Approximately 87−94% of phenytoin in the bloodstream is bound to plasma protein and is pharmacologically inactive, while unbound (free) phenytoin has pharmacological activity. If phenytoin is administered in combination with various other drugs or is administered to patients with complications, the free phenytoin level may increase to induce toxicity. From 70−80% of a dose of phenytoin undergoes hydroxylation in the liver and is metabolized to an inactive metabolite (5-hydroxyphenyl-5-phenylhydantoin, HPPH).[3)] Then 90% of HPPH is excreted in the urine as a glucuronic acid conjugate of parahydroxyphenyl hydantoin, while approximately 5% is excreted without conjugation.
Because the effective concentration range is narrow and there is considerable individual variation of drug absorption, metabolism, and excretion, monitoring the blood level of phenytoin is essential for adjusting the dosage during treatment.[1),4),5)] Toxicity of phenytoin is dose-dependent and mainly affects the central nervous system. The chief symptoms of toxicity include nystagmus, ataxia, sleepiness, diplopia, and visual disturbance. Seizures may also be caused. As symptoms of toxicity not involving the central nervous system, gingival hyperplasia, anemia, and osteomalacia may occur during long-term phenytoin therapy. As symptoms of toxicity that are not necessarily dose-dependent, rash, adenopathy, hepatitis, blood dyscrasias, and systemic lupus erythematosus have also been reported.
Monitoring the blood level of phenytoin is useful for adjusting the dosage so that the optimum clinical response can be obtained without symptoms of toxicity.

Assay Principle

1. Assay Principle

When a certain amount of anti-phenytoin antibody is added and reacted with a sample, consumption of the antibody depends on its content in the sample. When phenytoin-coated latex is added, residual anti-phenytoin antibody reacts with the latex and forms aggregates.
Since the extent of aggregation depends on the phenytoin concentration in the sample, the phenytoin concentration can be determined by measuring aggregation as the change of absorbance.

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

Unreacted anti-phenytoin antibody + Phenytoin-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 4 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.[6)]
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), formazin turbidity (up to 2500 FTU), or rheumatoid factors (up to 200IU/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 phenytoin and cross-reactivity between phenytoin and other drugs.

Substance	Concentration tested (μg/mL)	Cross-reactivity (%)
5-(p-Methylphenyl)-5-phenylhydantoin	500	5.60
Amitriptyline	3000	0.01
Amobarbital	100	0.05
Carbamazepine	500	0.08
Carbamazepine-10,11-epoxide	1000	0.06
Clorazepate	2000	0.03
Chlordiazepoxide	2000	0.01
Chlorpromazine	2500	0.00
Diazepam	2000	0.02
Ethosuximide	1000	0.02
Ethotoin	1000	0.17
Glutethimide	1000	0.00
5-(p-Hydroxyphenyl)-5-phenylhydantoin (HPPH)	500	2.46
Hydantoin	2000	0.02
Imipramine	4000	0.01
Mephenytoin	3000	0.04
Mephobarbital	1000	0.07
Methsucimide	5000	0.01
Oxaprozin	500	0.10
2-Phenyl-2-ethylmalonamide	1000	0.01
Pentobarbital	1000	0.03
Phenobarbital	2000	0.00
Phensuximide	2000	0.04
p-Hydroxyphenobarbital	2000	0.00
Primidone	1000	0.04
Promethazine	1500	0.01
Secobarbital	2000	0.02
Valproic acid	7000	0.00

3. Others

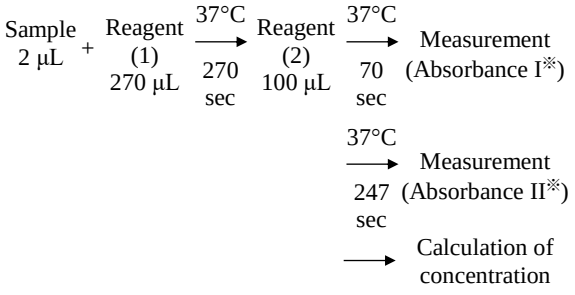
1) Always use TDM Calibrator for Nanopia for calibration.
2) Precautions for assay range
If the concentration of a target substance in the sample exceeds the measurement range, dilute the sample with a separately sold diluent (manufactured by SEKISUI MEDICAL CO., LTD.), and perform re-measurement.

Dosage/Administration (Assay Procedure)

1. Preparation of reagents

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

2. Assay Procedure



※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 therapeutic concentration of phenytoin has been reported to be 10−20 μg/mL in adults and children, 8−15 μg/mL in newborns.[7)] Assay results should be assessed while taking into consideration the patient's clinical findings and other laboratory data.
It is recommended that the blood concentration of phenytoin 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 of the standard solution (0 μg/mL) per minute is 0.05−0.21.
2) The ratio between the change of absorbance per minute with the standard solution (0 μg/mL) and that with another standard solution (2.5 μg/mL) is 60−95%.

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[8)]: (On Hitachi 7170S

automated analyzer)
0.7–40 μg/mL

5. **Correlation**[8)]
   1) Serum N=65 r=0.997 y=1.03x+0.06
   Control method: Approved in vitro diagnostic (enzyme immunoassay)
   2) Plasma N=98 r=0.995 y=0.95x+0.16
   Control method: Approved in vitro diagnostic (enzyme immunoassay)
6. **Standard Material**
   Phenytoin (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 PHT Antibody Solution 1 and PHT 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 PHT Antibody Solution 1 and PHT 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: 19 months from the date of manufacture (The expiration date is printed on the outer package.)

Packaging

Name		Package
Nanopia TDM Phenytoin	PHT Antibody Solution 1	1 × 54 mL
	PHT Latex Reagent 2	1 × 20 mL

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

References **

1) Buchthal F., Lennox-Buchthal M. A. : Diphenylhydantoin : Relation of anticonvulsant effect to concentration in serum, in Woodbury, D. M., Penry J. K., Schmidt K. P. （eds.）: Antiepileptic Drugs, Raven Press, New York, pp. 193−209, 1972.
2) Finn A. L., Olanow C. W.: Phenytoin: Therapeutic use and serum concentration monitoring, in Taylor, W. J., Fin, A. L. (eds.): Individualizing Drug Therapy : Practical Application of Drug Monitoring, Vol 2, Gross, Townsend, Frank, Inc., New York, pp. 63−85, 1981.
3) Jusko W. J.: Bioavailability and disposition kinetics of phenytoin in man, in Kellaway, P., Petersen, I.（eds.): Quantitative Analytic Studies in Epilepsy, Raven Press, New York, pp. 115−136, 1976.
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5) Buchthal F., Svensmark O.: Serum concentrations of diphenylhydantoin （ Phenytoin ） and phenobarbital and their relation to therapeutic and toxic effects, Psychiat. Neurol. Neurochir., 74: 117−136, 1971.
6) Sawada T. et al.: J Med Pharm Sci, 51(1), 131−141, 2004.
7) The Japanese Society of Therapeutic Drug Monitoring. [Guideline for Therapeutic Drug Monitoring (TDM) for Antiepileptic Drug 2018]. Tokyo: Kanehara Shuppan; 2018. pp. 8-9, pp. 32-39.
8) In house data, SEKISUI MEDICAL CO., LTD.

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