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

In Vitro Diagnostics
Marketing Approval No. 21600AMZ00593000

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This package insert must be read carefully prior to use.

Urea nitrogen assay kit
(Classification No.: 42849002)

QUALIGENT UN

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. For the effects of an administered drug on the measured value, carefully read the Precautions for Use in the package insert of the drug, especially the section about the effects on laboratory test results. Please also read carefully the "2. Interfering Substances," in the "Procedural Precautions" section, as well as "2. Precautions for Assessment" in the "Assessment of Assay Results" section, of this package insert.
4. 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.
5. 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.
6. 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.
7. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components) *

Component: Ingredients

UN Enzyme Solution 1:
Nicotinamide adenine dinucleotide (reduced form)
Sodium 2-ketoisohexanoate
Leucine dehydrogenase

UN Enzyme Solution 2:
Nicotinamide adenine dinucleotide (reduced form)
Sodium 2-ketoisohexanoate
Urease
Leucine dehydrogenase

Intended Use

Measurement of urea nitrogen in serum, plasma or urine

Urea is the final product of protein metabolism. It is released into the blood from the liver and then eliminated from the body via the kidneys. In patients with nephropathy, the blood urea nitrogen level increases because of impaired excretion. Abnormal values are seen in patients with heart disease, liver disorder, dehydration, or hemorrhage (especially gastrointestinal hemorrhage), as well as those with nephropathy.

Assay Principle

1. Assay Principle

First reaction:
Ammonia in the sample reacts with 2-ketoisohexanoic acid and reduced nicotinamide adenine dinucleotide (NADH) in the presence of leucine dehydrogenase (LED). As a result, leucine and oxidized nicotinamide adenine dinucleotide (NAD) are produced, and the absorbance at 340 nm decreases.
Second reaction:
If urease is added after completion of the first reaction, ammonia and carbonic acid are produced from urea. Ammonia produced by urease reacts with 2-ketoisohexonic acid and reduced nicotinamide adenine dinucleotide in the presence of leucine dehydrogenase. As a result, leucine and nicotinamide adenine dinucleotide are produced, and the absorbance at 340 nm decreases.
The concentration of urea nitrogen is determined by subtracting the change of absorbance with the first reaction from the change with the second reaction.

First reaction:

$$NH_3 + \text{2-Ketoisohexanoic acid} + NADH \xrightarrow{LED} \text{Leucine} + H_2O + NAD$$

Second reaction:

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_3 + H_2CO_3$$

$$NH_3 + \text{2-Ketoisohexanoic acid} + NADH \xrightarrow{LED} \text{Leucine} + H_2O + NAD$$

2. Features

1) The influence of endogenous ammonia can be avoided.
2) Analytical results are hardly affected by hemolysis or bilirubin.

Procedural Precautions **,*

1. Properties of Samples and Sampling Methods

1) Samples
Serum, plasma (heparin plasma, EDTA plasma and citrated plasma) and urine may be used.
When urine is measured, dilute it 1:21 with physiological saline in advance or measure it by using separately designated parameters.
2) Storage of samples[2)]
If the isolated serum or plasma sample cannot be tested on the same day, specimens

should be stored as follows:
2–10°C: for tests within 1 week
≤ -20°C: for tests after more than 1 week
Bring samples to room temperature (15–30°C) before use.
Urine samples should be tested on the same day.

2. Interfering substances

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 3000 FTU), or ammonia (up to 80 mg/dL).

3. Others

1) Always use Serum Multicalibrator (SEKISUI), Seronorm Multicalibrator, Seronorm Human or Anaserum UN standard solution for calibration.
2) Precautions for assay range
If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

Dosage/Administration (Assay Procedure) ** *

1. Preparation of reagents

Reagent (1): UN Enzyme Solution 1 is ready to use.
Reagent (2): UN Enzyme Solution 2 is ready to use.

2. Assay Procedure

This product is compatible with Hitachi 9000 series and LABOSPECT series automated analyzers. Assay procedure is indicated below.

Sample 5.0 µL + Reagent (1) 100 µL → (37°C, 59–293 sec) → Measurement (absorbance I※)
Reagent (2) 33 µL → (37°C, 65–299 sec) → Measurement (absorbance II※)
→ Calculation of concentration

※Absorbances I and II: The difference in absorbance between 700 nm and 340 nm.
Calibration material: Serum Multicalibrator (SEKISUI), Seronorm Multicalibrator, Seronorm Human or Anaserum UN standard solution (Manufacture's assigned value)
Reagent blank: Purified water or saline

Assessment of Assay Results ** *

1. Reference standard range[3)]

In blood: 8–20 mg/dL[3)] (Equivalent to urea of 17 - 43 mg/dL)
(Within the JCCLS common standard)

2. Precautions for Assessment

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) Reagent blank: change in absorbance being equal to or lower than 0.010/min
2) Sensitivity: The change of absorbance is 0.035–0.065/min per 50 mg/dL of urea nitrogen.

2. Accuracy: 90–110% of the expected assay value

3. Within-run Reproducibility:

Coefficient of variation ≤ 5%
(Test methods used for 1. –3. are in-house methods.)

4. Measurement Range[4)]: (On a Hitachi 9000 series automated analyzer)

0.6–300 mg/dL

5. Correlation[4)]

1) Serum N=63 r=0.999 y=0.99x–0.08
Control method: Approved in vitro diagnostic (urease-GLDH method)
2) Plasma N=88 r=0.999 y=0.98x –0.29
Control method: Approved in vitro diagnostic (urease-GLDH method)
3) Urine N=50 r=0.999 y=0.98x –2.06
Control method: Approved in vitro diagnostic (urease-GLDH method)

6. Standard Material

SRM912 (NIST)

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 UN Enzyme Solution 1 and UN Enzyme Solution 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 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 antisepticagent in the UN Enzyme Solution 1 and UN Enzyme Solution 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

1) Do not use the containers for other purposes.
2) Do not take apart the reagent cartridge before.

Storage and Shelf Life *

1. Storage temperature: 2–10°C
2. Shelf life: 1 year from the date of manufacture
(The expiration date is printed on the outer package.)

Packaging *

Name	Package		
QUALIGENT UN	Set (Cassette for Hitachi 9000 series)	UN Enzyme Solution 1 1 × 15.0 mL	× 2
		UN Enzyme Solution 2 1 × 5.0 mL	
	L set (Set for Hitachi LABOSPECT series)	UN Enzyme Solution 1 1 × 46 mL	× 2
		UN Enzyme Solution 2 1 × 16 mL	

References **

1) Morishita Y. et al.: J Jpn Soc Clin Chem 25, Enlarged No. 3, 30c, 1996. Japanese.
2) Sasaki M1. et al.: Sampling of constituents of the human body, Tokyo: Kodansha; 1972. Japanese.
3) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 35th ed. Tokyo: Kanehara Shuppan; 2020. Japanese.
4) SEKISUI MEDICAL CO., LTD. In house data. Japanese.

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