



**Wako**

# Autokit Glucose

**Enzymatic Method (Mutarotase-GOD)**

For the quantitative determination of glucose

## Summary and explanation of the test

Muller first demonstrated that  $\beta$ -glucose oxidase promoted the oxidation of glucose by molecular oxygen to gluconic acid. Franke and Lorenz discovered that hydrogen peroxide is simultaneously produced in this reaction. In 1956, first Keston and then Teller introduced the enzymatic methods for the determination of glucose, combining glucose oxidase (GOD), peroxidase (POD) and an oxygen acceptor (chromogen), which had high specificity and simplicity. There were drawbacks with this method, such as interferences from the reducing substances in samples and a potential for carcinogenesis (o-tolidine and o-dianisidine).

Several modifications and improvements, including the use of 4-aminoantipyrine as chromogen, introduced by Trinder in 1969, have been reported.

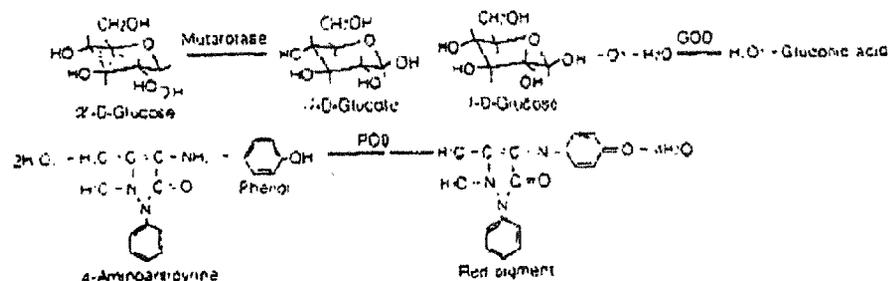
## Principle of the method

The equilibrium of D-glucose in solution is maintained in the ratio of  $\alpha$ -D-glucose 36.5% and  $\beta$ -D-glucose 63.5%. GOD reacts only with  $\beta$ -D-glucose.

When a test sample is allowed to react with the reagent,  $\alpha$ -D-glucose existing in the sample is converted rapidly to the  $\beta$ -isomer by the action of mutarotase and is then oxidized by GOD to produce hydrogen peroxide. In the absence of mutarotase, the reaction proceeds slowly because  $\beta$ -D-glucose is first consumed by GOD as  $\alpha$ -D-glucose is gradually converted to  $\beta$ -D-glucose. When mutarotase is added,  $\alpha$ -D-glucose is rapidly converted to  $\beta$ -D-glucose, so that GOD action is facilitated.

The hydrogen peroxide produced induces oxidative condensation between phenol and 4-aminoantipyrine in the presence of POD, so that a red color is produced. The amount of glucose contained in the test sample is determined by measuring the absorbance of the red color.

## Reactions



## Reagents

### Contents and storage conditions

	(996-90901)	(994-90902)
① Buffer Solution	2 bottles × 150 mL	6 bottles × 350 mL

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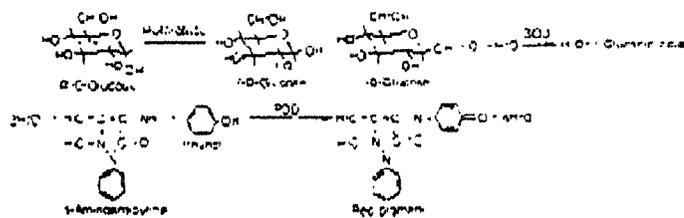
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## Reactions



## Reagents

### Contents and storage conditions

	(996-90901)	(994-90902)
① Buffer Solution	2 bottles × 150 mL	6 bottles × 350 mL
② Color Reagent	2 bottles × for 150 mL	6 bottles × for 350 mL
Standard I	1 bottle × 10 mL	1 bottle × 25 mL
Standard II	1 bottle × 10 mL	1 bottle × 25 mL

Store all reagents at 2–10°C

### Ingredients

Buffer Solution (pH 7.1)	Phosphate buffer Phenol	60 mmol/L 5.3 mmol/L
Color Reagent (When reconstituted)	Mutarotase	0.13 U/mL
	Glucose oxidase	9.0 U/mL
	Peroxidase	0.65 U/mL
	4-Aminantipyrine	0.50 mmol/L
Standard I	Ascorbate oxidase	2.7 U/mL
	Glucose	200 mg/dL
Standard II	Glucose	500 mg/dL

## Warnings and precautions

For *in vitro* Diagnostic Use.

Not to be used internally in humans or animals.

Do not use reagents past the expiration date stated on each reagent container label.

Do not use the preparations, test solutions and reagents for any other purpose than described herein.

## Physical or chemical indications of instability

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

## Instruments

This reagent is designed to be used on commercially available spectrophotometers or on RA-1000 analyzer. Refer to the operating manual for a description of instrument operation and specifications.

## Specimen collection and preservation

### Serum and plasma

Samples can be stored for 1 day at 25°C or 3 days at 2–10°C without significant effect on the measured values.

The whole blood glucose is consumed by blood cells during blood coagulation and serum separation. Therefore, the separation of blood cells should be done as quickly as possible after the blood collection.

Sodium fluoride, an inhibitor of glycolysis, does not affect the measurement of glucose.

Anticoagulants such as heparin, oxalate, citrate and EDTA do not influence measurements when they are employed in their usual amounts.

Ascorbic acid introduces a slight negative error in the measurement.

Bilirubin introduces a slight negative error in the measurement.

Hemolysis introduces a slight positive error in the measurement.

## Reagent preparation

### Working solution

Dissolve the whole contents of one bottle (for 150 mL or for 350 mL) of Color Reagent ② in one bottle (150 mL or 350 mL) of Buffer Solution ①. This solution is stable for one month at 2–10°C.

## Manual procedure

### Materials supplied

Refer to the section entitled "Reagents".

### Materials required but not supplied

Pipettes

Water bath or heating block capable of maintaining 37°C, and spectrophotometer.

### Test procedure

Wavelength: 505 nm\*\*

Light path: 1 cm

Temperature: 37°C

	Sample (S)	Standard (Std)	Blank (Bl)
Pipette into a cuvette			
Sample (mL)	0.02	—	—*2
Standard I or II (mL)	—	0.02	—
Working solution (mL)	3.0	3.0	3.0
Mix well, incubate for 5 min, and measure the absorbance of S (A <sub>S</sub> ) and Std (A <sub>Std</sub> ) against Bl (A <sub>Bl</sub> ) at 505 nm.			

1. Accurately pipette 0.02 mL of sample or standard into the cuvettes (test tubes).

2. Add 3.0 mL of Working solution.

3. Mix, incubate for 5 min, and measure the absorbance of Sample (A<sub>S</sub>) and Standard (A<sub>Std</sub>) against Blank (A<sub>Bl</sub>) at 505 nm.

\*1 When measure with two wavelengths,  $\lambda_1/\lambda_2 = 505/600$  nm.

\*2 The omission of 0.02 mL of water does not significantly affect the absorbance measured.

### Concentration in the test (Manual procedure)

60 mmol/L Phosphate buffer, 5.3 mmol/L Phenol, 0.13 U/mL Mutarotase, 9.0 U/mL GOD, 0.65 U/mL POD, 0.50 mmol/L 4-Aminantipyrine and 2.7 U/mL AOD.

### Results (Manual procedure)

#### Calculation

$$\text{Glucose (mg/dL)} = \frac{A_S}{A_{Std}} \times C_{Std}$$

A<sub>S</sub> = Absorbance of sample

A<sub>Std</sub> = Absorbance of Standard I or II

C<sub>Std</sub> = Concentration of Standard I or II in mg/dL

#### Limitations of the procedure (Manual procedure)

When glucose value exceeds 700 mg/dL, dilute sample 1 + 1 with saline or distilled water, repeat assay and multiply result by 2.  
See Technical Information.

#### Automated procedure (RA-1000)

##### Materials supplied

Refer to the section entitled "Reagents"

##### Materials required but not supplied

RA-1000 analyzer

##### Test procedure (RA-1000)

CHEM ±	*	DECIMAL	0
NAME	1 GLC	RBL LOW	0.0
IMNOASSY	0	RBL HI	0.1
TYPE	2	RANGE LO	0
INVERSE	0	RANGE HI	500
% SMP VOL	6 (3 µL)	CAL FACT	—
FILTER P	4 (500 nm)	NORMAL L	70
DELAY	5:00	NORMAL H	110
% RGT VOL	70 (350 µL)	SLOPE	1.0
2ND RGT	0	INTERCPT	0.0
UNIT	2 (MG/DL)	1=ACCEPT	1
UNIT FAC	1.0		

##### Concentration in the test (RA-1000)

60 mM/L Phosphate buffer, 5.3 mM/L Phenol, 0.13 U/mL Mucicase, 9.0 U/mL GOD, 0.65 U/mL POD, 0.50 mM/L 4-Aminoantipyrene and 2.7 U/mL AOD

##### Results (RA-1000)

The final results are automatically calculated and printed in concentration.

##### Limitations of the procedure (RA-1000)

When glucose value exceeds 700 mg/dL, dilute sample 1 + 1 with saline or distilled water, repeat assay, and multiply result by 2.  
See Technical Information.

##### Expected values

Serum 70 - 110 mg/dL

Since expected values are affected by age, sex, diet, geographical location and other factors, each laboratory should establish its own expected values for this procedure.

##### Performance characteristics

Refer to Technical Information.

Accuracy: See Technical Information.

Precision: See Technical Information.

Sensitivity: The theoretical sensitivity of this method expressed as absorptivity is 35.9 U/gm·cm at 505 nm.

Specificity: The specificity of the Autokit Glucose test method was determined by analysis of serum samples which contained the addition of potential interfering substances.  
See Technical Information.

##### Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control sera both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for the controls should fall within the manufacturer's accepted ranges.

If values are to be established for unassayed control sera, the laboratory should assay each serum a sufficient number of times to generate a valid mean and acceptable range.

#### Additional recommended products

Code No 410-00101 Control Serum I (10 x 10 for 5mL) Normal  
Code No 416-00201 Control Serum I (10 x 10 for 5mL) Abnormal

#### References

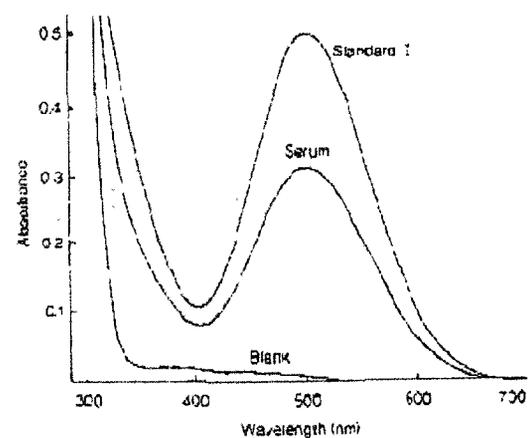
1. Miwa, I., Okuda, J., Maeda, K. and Okuda, G. Clin. Chim. Acta, 37, 538-540 (1972).
2. Okuda, J. and Miwa, I. Protein, Nucleic acid and Enzyme 17, 216-224 (1972).
3. Okuda, J., Miwa, I. and Maeda, K. Japanese Journal of Clinical Chemistry 2, 289-296 (1973).
4. Tinnoc, P. Ann. Clin. Biochem., 6, 24 (1969).
5. Okuda, J., Miwa, I., Maeda, K. and Tokui, K. Carbohydrate Research 58, 267 (1977).
6. Miwa, I., Toyoda, Y. and Okuda, J. Journal of Medical Technology, 22, 1232 (1978).
7. Kingsley, G. P. and Gatchell, G. Clin. Chem. 6, 466 (1960).

#### Ordering information

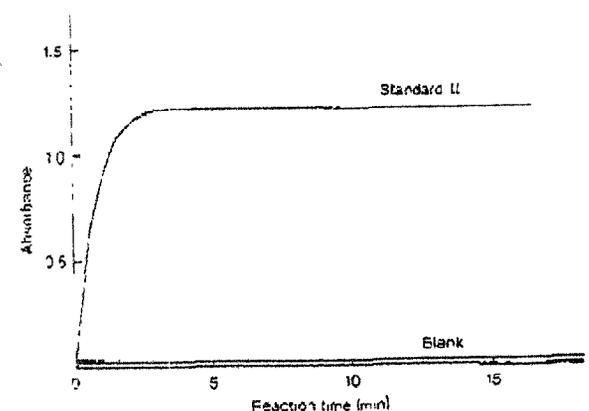
Code No.	Products	Package
993-90901	Autokit Glucose	2 x 150mL
994-90902	Autokit Glucose	6 x 350mL

#### Technical Information

##### Absorption spectrum

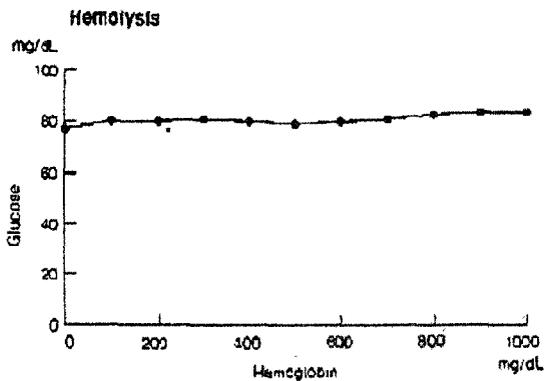
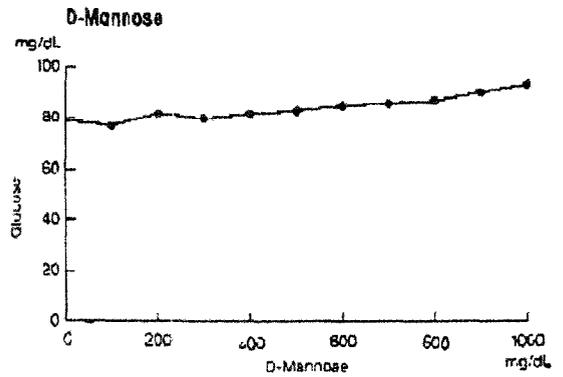
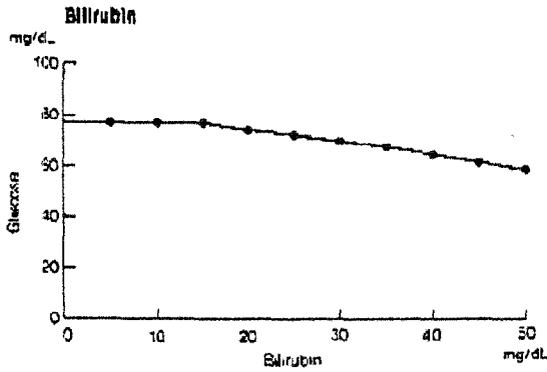
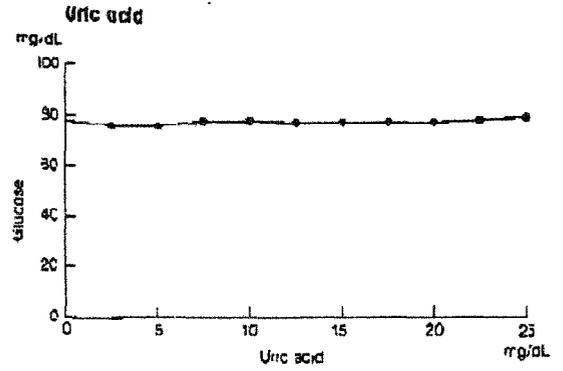
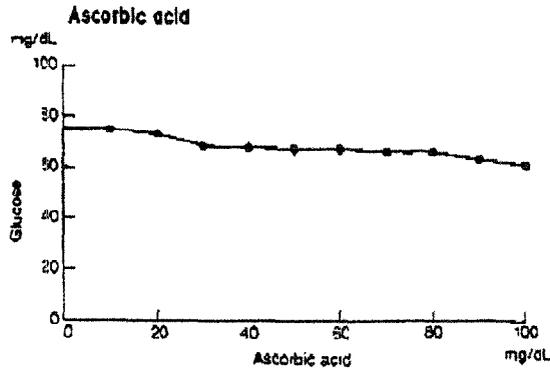


##### Reaction time courses





**Specificity**



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