

Glycerol, Free (Enzymatic Color Test)

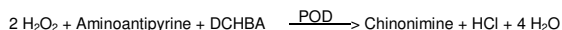
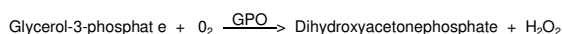
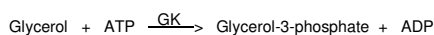
Cat.No	Package Size
138 000	4 x 50 ml R1 / 2 x 20 ml R2 + 2 x Standard

METHOD

Enzymatic Color Test, Endpoint.

PRINCIPLE

Enzymatic determination of glycerol according to the following reactions:



The Glycerol is oxidized under the catalytic effect of GK and GPO to yield Dihydroxyacetonephosphate and Hydrogenperoxide.

The latter is oxidizing (catalyzed through Peroxidase) Aminoantipyrine und Dichlorohydroxybenzoic Acid (DCHBA) to form a red colored Chinonimine which is measured photometrically, and is proportional to the concentration of the Glycerol.

REAGENT

Composition (concentrations in the test)

Reagent R1:

Pipes Buffer	pH 7,5	50 mmol/l
DCHBA		0,2 mmol/l
ATP		2 g/l
Glycerol-3-phosphateoxidase (GPO)		≥ 1,5 kU/l
Peroxidase (POD)		≥ 2 kU/l

Reagent R2:

Glycerokinase (GK)		4 kU/l
Pipes Buffer	pH 7,5	50 mmol/l
Aminoantipyrine		2 mmol/l

Standard:	Glycerol	0,56 mmol/l
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Precautions

- For *in vitro* diagnostic use only.
- The reagents contain less than 0,95 g/l NaN₃. Do not swallow and keep away from skin and mucous membranes.

Stability

When stored at 2-8° C and protected from light, the reagent is stable up to the expiry date printed on the labels.

Do not freeze reagents !

Preparation and stability of Working reagents

Reagent R1 and **Standard** are ready for use.

Reagent R3 : Prepare from 50 ml R1 und 10 ml R2.

Let stand for 1 hour before using it .

Stability: 6 months at 2 – 8 °C

SAMPLES

Serum, Heparin plasma , EDTA-Plasma.

Stability:	2 days	at	20 - 25 °C
	7 days	at	4 - 8 °C
	> 1 year	at	< -20 °C

Disregard contaminated material !

REFERENCE VALUES

Serum / Plasma

< 10 mg/dL (0.114 mmol/l)

ANALYTICAL PROCEDURE

Applications are available on request.

Wavelength :	550 nm, 546 nm
Temperature :	37 °C (or 20 – 25 °C)
Cuvette :	1 cm light path

Measure against water (increasing absorbance A)

	Reaction I	Reaction II
Sample(S)/Control(Ctr)	80 µl	80 µl
Standard(Std)		
Reagent R1	1000 µl	-
Reagent R3	-	1000 µl

Mix well , incubate for 10 min at 20-25 °C or 5 min at 37 °C , then measure absorbances A of (I) and (II)

In the same way measure A's of reagent blanks **RB(I)** and **RB(II)** by using dist. water instead of S/Std/Ctr

CALCULATION

$$\text{Free Glycerol} = 0,56 \times \frac{(A_S(II) - A_S(I)) - (A_{RB}(II) - A_{RB}(I))}{(A_{Std}(II) - A_{Std}(I)) - (A_{RB}(II) - A_{RB}(I))}$$

QUALITY CONTROL

For Quality Control Greiner normal and abnormal controls Unitrol-I and Unitrol-II are recommended

PERFORMANCE DATA

are available from Greiner on request

INTERFERENCES

Interferences are found according to literature and the following limits:

Bilirubine	> 5 mg/dL
Hemoglobin	>500 mg/dL
Ascorbic Acid	> 5 mg/dL