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2,3-Diphosphoglycerate (2,3-DPG)

UV-test for the determination of 2,3-DPG in blood research samples

Cat. No. 10 148 334 001

Test-Combination for approx. 30 determinations

Version 23

Content version: April 2018

Store at +2 to +8°C

Product overview

Contents

The Test-Combination contains:

Bottle	Label	Contents
1	Triethanolamine buffer	▪ 70 ml ▪ 48 mM Triethanolamine buffer, pH 7.6, 5.2 mM EDTA, 5.3 mM $MgCl_2$ ▪ ready-to-use
2	ATP and NADH	▪ 2 bottles ▪ containing approx. 24 mg ATP and approx. 8.2 mg NADH each.
3	PGM, PGK,GAP-DH, TIM, GDH	▪ Lyophilizate ▪ containing approx. 25 U PGM, 1600 U PGK, 25 U GAP-DH, 870 U TIM, and 230 U GDH
4	Phosphoglycerate mutase (PGM)	▪ Lyophilizate ▪ 620 U PGM
5	Glycolate-2 -phosphate, tricyclohexylammonium salt	16.5 mg

Test principle (1,2)

2,3-DPG is split by the side activity of phosphoglycerate mutase (PGM), activated with glycolate-2-phosphate, to form phosphoglycerate (PG) (1).

(1) 2,3-DPG $\xrightarrow[\text{glycolate-2- phosphate}]{\text{PGM}}$ PG + P_i

Both, 2-PG and 3-PG can be formed. 2-PG is isomerised by reaction (2) into 3-PG. 3-PG is converted by phosphoglycerate kinase (PGK) (3), glyceraldehyde-3-phosphate dehydrogenase (GAP-DH) (4), triosephosphate isomerase (TIM) (5) and glycerol -3-phosphate dehydrogenase(GDH) (6), 2 moles of NADH being oxidized per mole of 2,3-DPG.

(2) 2-PG $\xrightarrow{\text{PGM}}$ 3-PG

(3) 3-PG + ATP $\xrightarrow{\text{PGK}}$ 1,3-DPG + ADP

(4) 1,3-DPG + NADH + H^+ $\xrightarrow{\text{GAP-DH}}$ glyceraldehyde-3-P + NAD^+ +Pi

(5) Glyceraldehyde-3-P $\xrightarrow{\text{TIM}}$ dihydroxyacetone-P

(6) Dihydroxyacetone-P + NADH + H^+ $\xrightarrow{\text{GDH}}$ glycerol-3-P + NAD^+

Reactions (2)-(6) are carried out first of all to eliminate any substrates present in the assay mixture. The quantity of PGM is so small that reaction (1) will not yet start.

Application

Determination of 2,3-Diphosphoglycerate in blood in the range of 0.02–0.15 µmol in life science research applications.

Number of tests

Test-Combination for approx. 30 determinations

Preparation of working solutions

Please refer to the following table

Bottle	Preparation	Final concentration
2	Dissolve contents of bottle 2 in 1 ml double dist. water.	40 mM ATP, 9.6 mM NADH
3	Dissolve contents of bottle 3 in 1.75 ml Triethanolamine buffer (bottle 1).	14×10^3 U/l PGM 94×10^4 U/l PGK 14×10^3 U/l GAP-DH 50×10^4 U/l TIM 13×10^4 U/l GDH
4	Dissolve contents of bottle 4 in 0.7 ml Triethanolamine buffer (bottle 1).	88×10^4 U/l PGM
5	Dissolve contents of bottle 5 in 0.7 ml double dist. water.	48 mM Glycolate-2-phosphate

Storage/stability

Bottle	Contents	Storage/stability
1	Triethanolamine buffer	1 year at +2 to +8°C
2	ATP and NADH	10 days at +2 to +8°C
3	PGM, PGK,GAP-DH, TIM, GDH	3 weeks at +2 to +8°C
4	Phosphoglycerate mutase (PGM)	3 weeks at +2 to +8°C
5	Glycolate-2-phosphate	6 weeks at +2 to +8°C

Assay procedure- Determination in blood

Additional reagents required

- Perchloric acid, approx. 0.6 M
- Potassium carbonate solution, approx. 2.5 M.

Sample preparation

Please refer to the following table.

Note: When the blood sample has been collected the 2,3-DPG content within will change rapidly. For this reason, the deproteinization procedure described in the table below should be carried out immediately.

Step	Action
1	Collect blood from veins in ice-cooled heparinized test tubes. ***Note:*** Carry out deproteinization immediately.
2	Pipette into a 10 ml centrifuge tube 5 ml Perchloric acid, approx. 0.6 M (ice cooled).
3	Add 1 ml blood and mix. ***Note***: Flush pipette by repeated filling and emptying.
4	Centrifuge mixture at 5,000 rpm for 10 min.
5	Take 4 ml of the clear, colorless supernatant and neutralize with 0.5 ml 2.5 M Potassium carbonate.
6	Keep for at least 60 min in an ice-bath.
7	Remove perchlorate precipitate by filtration or centrifugation in the cold. Use 0.1 ml of the supernatant for the assay. ***Note***: 2,3-DPG is stable for at least 1 day in the neutralized extracts.

Assay protocol

Please refer to the following table.
Note: The determination of the blank (once per series) is necessary if extremely high precision is required for scientific investigations.

Step	Action
1	Pipette into glass cuvettes (1 cm light path) the following solutions: **Solution** / **Blank** / **Sample** Triethanolamin buffer (bottle 1) / 2.00 ml / 2.00 ml Solution 2 / 0.05 ml / 0.05 ml Solution 3 / 0.05 ml / 0.05 ml Sample (neutralized) / — / 0.1 ml Double dist. water / 0.1 ml / — ***Note***: Solution 1, 2 and 3 may be mixed in the ratio indicated above and the sum of their volumes pipetted. (Stable for 3 days at +4°C).
2	Mix and allow to stand at +20 to +25°C, read absorbance A_1, after the reaction has stopped (approx. 5 min). ***Note***: Wavelength: 340 nm, Hg 365 nm or Hg 334 nm.
3	Add to the cuvettes the following solutions: **Solution** / **Blank** / **Sample** Solution 4 / 0.02 ml / 0.02 ml Solution 5 / 0.02 ml / 0.02 ml Final volume / 2.24 ml / 2.24 ml ***Note:*** Solution 4 and 5 may be mixed in the ratio indicated above and the sum of their volumes pipetted. (Stable for 3 days at +4°C).
4	Mix and wait for the end of the reaction (approx. 25 min). Read absorbance A_2. ***Note***: Wavelength: 340 nm, Hg 365 nm or Hg 334 nm.

Calculation

ΔA

$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank}$
Note: The maximum difference in absorbance should not exceed $\Delta A_{365\ nm} = 0.400$ (or $\Delta A_{334,\ 340\ nm} = 0.720$).

Otherwise use 0.05 ml of sample and 2.05 ml of Solution 1. In this case multiply ΔA by the factor 2.

Concentration of 2,3 DPG

$$c = \frac{V \times MW \times F}{\epsilon \times d \times v \times 1000 \times 2} \times \Delta A \text{ [g/l blood]}$$

V = assay volume [ml] = 2.24 ml
v = sample volume [ml] = 0.1 ml
MW = molecular weight of 2,3-DPG = 266.037 g/mol
d = light path [cm] = 1 cm
ϵ = absorption coefficient of NADH at:
340 nm = 6.3[l × mmol^{-1} × cm^{-1}]
Hg 365 nm = 3.4[l × mmol^{-1} × cm^{-1}]
Hg 334 nm = 6.18[l × mmol^{-1} × cm^{-1}]
Dilution factor for blood (3) (80% water content)
F = 6.582.
It follows for the concentration of 2,3-DPG in blood:
$c = 21.68 \times \Delta A_{365\ nm}$ [mmol/l], or
$c = 5.767 \times \Delta A_{365\ nm}$ [g/l]
$c = 11.70 \times \Delta A_{340\ nm}$ [mmol/l], or
$c = 3.112 \times \Delta A_{340\ nm}$ [g/l]
$c = 11.93 \times \Delta A_{334\ nm}$ [mmol/l], or
$c = 3.173 \times \Delta A_{334\ nm}$ [g/l]

If the measurement is based on the volume of erythrocytes instead of blood, the result should be additionally multiplied by 100/HCR. (HCR = haematocrit value).

References

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Changes to previous version

Editorial changes.

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