

# 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

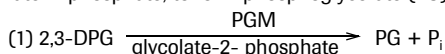
## Product overview

### Contents

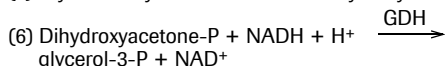
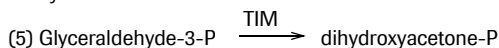
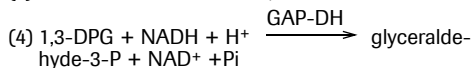
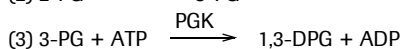
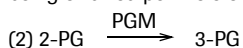
The Test-Combination contains:

Bottle	Label	Contents
1	Triethanolamine buffer	<ul style="list-style-type: none"> <li>70 ml</li> <li>48 mM Triethanolamine buffer, pH 7.6, 5.2 mM EDTA, 5.3 mM MgCl<sub>2</sub></li> <li>ready-to-use</li> </ul>
2	ATP and NADH	<ul style="list-style-type: none"> <li>2 bottles</li> <li>containing approx. 24 mg ATP and approx. 8.2 mg NADH each.</li> </ul>
3	PGM, PGK, GAP-DH, TIM, GDH	<ul style="list-style-type: none"> <li>Lyophilizate</li> <li>containing approx. 25 U PGM, 1600 U PGK, 25 U GAP-DH, 870 U TIM, and 230 U GDH</li> </ul>
4	Phosphoglycerate mutase (PGM)	<ul style="list-style-type: none"> <li>Lyophilizate</li> <li>620 U PGM</li> </ul>
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).



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.



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 <sup>3</sup> U/l PGM 94 × 10 <sup>4</sup> U/l PGK 14 × 10 <sup>3</sup> U/l GAP-DH 50 × 10 <sup>4</sup> U/l TIM 13 × 10 <sup>4</sup> U/l GDH
4	Dissolve contents of bottle 4 in 0.7 ml Triethanolamine buffer (bottle 1).	88 × 10 <sup>4</sup> 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. <b>Note:</b> 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. <b>Note:</b> 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. <b>Note:</b> 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: <table border="1"><thead><tr><th>Solution</th><th>Blank</th><th>Sample</th></tr></thead><tbody><tr><td>Triethanolamin buffer (bottle 1)</td><td>2.00 ml</td><td>2.00 ml</td></tr><tr><td>Solution 2</td><td>0.05 ml</td><td>0.05 ml</td></tr><tr><td>Solution 3</td><td>0.05 ml</td><td>0.05 ml</td></tr><tr><td>Sample (neutralized)</td><td>—</td><td>0.1 ml</td></tr><tr><td>Double dist. water</td><td>0.1 ml</td><td>—</td></tr></tbody></table> <p><b>Note:</b> 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).</p>	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	—
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	—																	
2	Mix and allow to stand at +20 to +25°C, read absorbance $A_1$ , after the reaction has stopped (approx. 5 min). <p><b>Note:</b> Wavelength: 340 nm, Hg 365 nm or Hg 334 nm.</p>																		
3	Add to the cuvettes the following solutions: <table border="1"><thead><tr><th>Solution</th><th>Blank</th><th>Sample</th></tr></thead><tbody><tr><td>Solution 4</td><td>0.02 ml</td><td>0.02 ml</td></tr><tr><td>Solution 5</td><td>0.02 ml</td><td>0.02 ml</td></tr><tr><td>Final volume</td><td>2.24 ml</td><td>2.24 ml</td></tr></tbody></table> <p><b>Note:</b> 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).</p>	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						
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																	
4	Mix and wait for the end of the reaction (approx. 25 min). Read absorbance $A_2$ . <p><b>Note:</b> Wavelength: 340 nm, Hg 365 nm or Hg 334 nm.</p>																		

## Calculation

### $\Delta A$

$$\Delta A = (A_1 - A_2)_{\text{sample}} - (A_1 - A_2)_{\text{blank}}$$

**Note:** The maximum difference in absorbance should not exceed  $\Delta A_{365 \text{ nm}} = 0.400$  (or  $\Delta A_{334, 340 \text{ nm}} = 0.720$ ).

Otherwise use 0.05 ml of sample and 2.05 ml of Solution 1. In this case multiply  $\Delta A$  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

$\epsilon$  = absorption coefficient of NADH at:

340 nm =  $6.3 [l \times \text{mmol}^{-1} \times \text{cm}^{-1}]$

Hg 365 nm =  $3.4 [l \times \text{mmol}^{-1} \times \text{cm}^{-1}]$

Hg 334 nm =  $6.18 [l \times \text{mmol}^{-1} \times \text{cm}^{-1}]$

Dilution factor for blood (3) (80% water content)

F = 6.582.

It follows for the concentration of 2,3-DPG in blood:

$c = 21.68 \times \Delta A_{365 \text{ nm}}$  [mmol/l], or

$c = 5.767 \times \Delta A_{365 \text{ nm}}$  [g/l]

$c = 11.70 \times \Delta A_{340 \text{ nm}}$  [mmol/l], or

$c = 3.112 \times \Delta A_{340 \text{ nm}}$  [g/l]

$c = 11.93 \times \Delta A_{334 \text{ nm}}$  [mmol/l], or

$c = 3.173 \times \Delta A_{334 \text{ nm}}$  [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

- 1 Ericson, A. & de Verdier, C. H. (1972) *Scand. J. Clin. Lab. Inv.* **29**, 85-90.
- 2 Michal, G. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.) pp. 1433-1438. Verlag Chemie, Weinheim, and Academic Press, New York.
- 3 Bergmeyer, H. U. (1977) in *Principles of Enzymatic Analysis* (Bergmeyer, H. U., ed.) pp. 217 and 236. Verlag Chemie, Weinheim and New York.
- 4 Müller-Wiefel, D. E. et al. (1978) *Monatsschr. Kinderheilkd.* **126**, 342-343.
- 5 Müller-Wiefel, D. E. et al. (1978) *Eur. J. Pediatr.* **128**, 103-111.

### Changes to previous version

Editorial changes.

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