

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

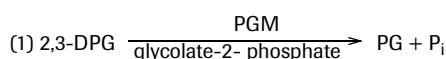
UV-test for the determination of 2,3-DPG in blood research samples

**Cat. No. 148 334**

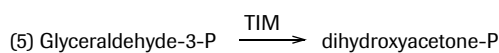
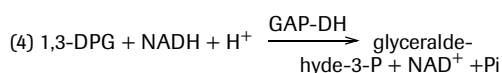
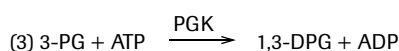
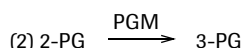
**Test-Combination for approx. 30 determinations**

## 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.

## The Test-Combination contains

1. Bottle 1 containing 70 ml of triethanolamine buffer, pH 7.6, with MgCl<sub>2</sub> and EDTA.
2. Two bottles 2 containing approx. 24 mg ATP and approx. 8.2 mg NADH each.
3. Bottle 3 containing approx. 25 U PGM, 1600 U PGK, 25 U GAP-DH, 870 U TIM and 230 U GDH as lyophilizate.
4. Bottle 4 containing 620 U PGM as lyophilizate.
5. Bottle 5 containing 16.5 mg glycolate-2-phosphate, tricyclohexylammonium salt.

## Preparation of solutions

- I. Use contents of bottle 1 undiluted.
- II. Dissolve contents of bottle 2 in 1.0 ml redist. water.
- III. Dissolve contents of bottle 3 in 1.75 ml buffer from bottle 1.
- IV. Dissolve contents of bottle 4 in 0.7 ml buffer from bottle 1.
- V. Dissolve contents of bottle 5 in 0.7 ml redist. water.

## Concentration of solutions

- I. 48 mM Triethanolamine buffer, pH 7.6, 5.2 mM EDTA, 5.3 mM MgCl<sub>2</sub>
- II. 40 mM ATP, 9.6 mM NADH
- III. 14 × 10<sup>3</sup> U/l PGM, 94 × 10<sup>4</sup> U/l PGK, 14 × 10<sup>4</sup> U/l GAP-DH, 50 × 10<sup>4</sup> U/l TIM, 13 × 10<sup>4</sup> U/l GDH
- IV. 88 × 10<sup>4</sup> U/l PGM
- V. 48 mM Glycolate-2-phosphate

## Stability of solutions

Solution I is stable for 1 year at 2-8°C.  
Solution II is stable for 10 days at 2-8°C.  
Solutions III and IV are stable for 3 weeks at 2-8°C.  
Solution V is stable for 6 weeks at 2-8°C.

## Additional reagents

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

## Determination in blood

Collect blood from veins in ice-cooled heparinized test tubes. Carry out deproteinization immediately.

## Deproteinization

pipette into a 10 ml-centrifuge tube and mix	
perchloric acid, approx. 0.6 mol/l (ice-cooled)	5.0 ml
blood	1.0 ml
flush pipette by repeated filling and emptying, centrifuge mixture. Take 4 ml of the clear supernatant and neutralize with 0.5 ml 2.5 M potassium carbonate. Keep for at least, 30 min in an ice-bath, filter off perchlorate precipitate or centrifuge off in the cold and use 0.1 ml of the supernatant for the assay.	

## Stability of sample

2,3-DPG is stable for at least 1 day in the neutralized extracts.

## Assay procedure

Wavelength: 340 nm, Hg 365 nm or Hg 334 nm  
Glass cuvette: 1 cm light path  
Measuring temperature: 20-25°C  
Final volume: 2.24 ml  
Read against air.

pipette into cuvette	blank	sample
solution I	2.00 ml	2.00 ml
solution II	0.05 ml	0.05 ml
solution III	0.05 ml	0.05 ml
sample (neutralized)	-	0.10 ml
water	0.10 ml	-
mix, allow to stand at 20-25°C, read absorbance A <sub>1</sub> after the reaction has stopped (approx. 5 min).		
solution IV	0.02 ml	0.02 ml
solution V	0.02 ml	0.02 ml
mix and wait for the end of the reaction (approx. 25 min). Read absorbance A <sub>2</sub> . $(A_1 - A_2)_{\text{sample}} - (A_1 - A_2)_{\text{blank}} = \Delta A$ .		

## Calculation

According to the general equation for calculating the concentration:

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

V = assay volume [ml]  
 v = sample volume [ml]  
 MW = molecular weight of 2,3-DPG  
 d = light path [cm]  
 ε = absorption coefficient of NADH at:  
 340 nm = 6.3 [l × mmol<sup>-1</sup> × cm<sup>-1</sup>]  
 Hg 365 nm = 3.4 [l × mmol<sup>-1</sup> × cm<sup>-1</sup>]  
 Hg 334 nm = 6.18 [l × mmol<sup>-1</sup> × cm<sup>-1</sup>]

Dilution factor for blood (3) (80% water content)  
 F = 6.582.

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

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

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

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

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

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

$$c = 3.173 \times \Delta A_{334 \text{ nm}} \text{ [g/l]}$$

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).

## Normal values

A concentration of 4.83 ± 0.15 mmol of 2,3-DPG/l erythrocytes was found in 10 men(). Erythrocytes of non-anemic children contain 5.29 ± 0.59 mmol 2:3-DPG/l erythrocytes (4).

## Notes

1. Solution I, II and III and solutions IV and V may be mixed in the ratio indicated above and the sum of their volumes pipetted. (Stable for 3 days at 4°C).
2. 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 DA by the factor 2.
3. After the blood sample has been taken the 2,3-DPG content changes quickly. For this reason, the above-mentioned deproteinization with perchloric acid should be carried out immediately.
4. The determination of the blank (once per series) is necessary if extremely high precision is required for scientific investigations.

## References

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E-mail Address	Country	E-mail Address	Country
argentina.biochem@roche.com	Argentina	Raitis@mvitros.lv	Latvia
biochem.au@roche.com	Australia	Sakkijha@rdleb.com	Lebanon
Gerhard.Muehlbauer@roche.com	Austria	Gintaras@eksm.lt	Lithuania
biochem.be@roche.com	Belgium	diagnostics@prophac.lu	Luxembourg
Valent@inbox.cil.bg	Bulgaria	Vcol@vol.net.mt	Malta
africhem@camnet.cm	Cameroon	Alouche.echo@dounia.net.ma	Morocco
biochem.ca@roche.com	Canada	biocheminfo.nl@roche.com	Netherlands
biochem.cn@roche.com	China	biochem.nz@roche.com	New Zealand
Info@medsell.com.cy	Cyprus	bofungwu@linkserve.com.ng	Nigeria
Bm-comp@bm-comp.cz	Czech Republic	biochem.se@roche.com	Norway
dk.biochem@roche.com	Denmark	biochem.pl@roche.com	Portugal
ou.melestrum@neti.ee	Estonia	Topdiag@fx.ro	Romania
pharscet@telecom.net.et	Ethiopia	biochem.sg@roche.com	Singapore
helsinki.biochem_diagnostics@roche.com	Finland	roche.diagnostics@siol.net	Slovenia
biochem.fr@roche.com	France	south_africa.biobffn@roche.com	South Africa
mannheim.biocheminfo@roche.com	Germany	biochem.es@roche.com	Spain
Bm_roche@hotmail.com	India	biochem.se@roche.com	Sweden
h.hajian@tebtech.com	Iran	BiochemInfo.CH@roche.com	Switzerland
tubanegin@istni.irost.com	Iran	Jean-Marie.kindbeiter@roche.com	Tunisia
Dyn@netvision.net.il	Israel	bmauae@emirates.net.ae	United Arab Emirates
it.biochem@roche.com	Italy	uk.biochem@roche.com	United Kingdom
biochemicals@rdj.co.jp	Japan	biochemts.us@roche.com	USA
pharmakp@net2000ke.com	Kenya	Mvalentiner@telcel.net.ve	Venezuela
Bmskorea@chollian.net	Korea	dusica@eunet.yu	Yugoslavia
react@ncc.moc.kw	Kuwait	biochemts.row@roche.com	All other countries

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**Argentina** 541 954 5555; **Australia** (02) 9899 7999; **Austria** (01) 277 87; **Belgium** (02) 247 4930; **Brazil** +55 (11) 3666 3565; **Bulgaria** +35929625408; **Cameroon** 237-370269; **Canada** (450) 086 7050; (800) 361 2070; **Chile** 00 56 (2) 22 33 737 (central) 00 56 (2) 22 32 099 (Exec); **China** 86 21 6427 5586; **Colombia** 0057-1-3412797; **Cyprus** +357-2-311362; **Czech Republic** (0324) 45 54, 58 71-2; **Denmark** +45 363 999 58; **Egypt** 20-2-3619047; **Estonia** 372-7-447600; **Ethiopia** 251-1-552799; **Finland** +358 9 525 333 66; **France** 04 76 76 30 87; **Germany** (0621) 759 8568; **Greece** +3 01 61 66 100; **Hong Kong** (852) 2485 7596; **India** +91-22-8379906; **Indonesia** 62 (021) 252 3820 ext. 755; **Iran** +98-21-8072374 / +98-21-8797027; **Israel** 972-6- 6380569; **Italy** 039 247 4109-4181; **Japan** 03 5443 5284; **Kenya** +254-2-750112; **Korea** 82-2-3471-6500; **Kuwait** +965-4837859; **Latvia** 371-787828309; **Lebanon** Fax: 00961-1-399667; **Lithuania** 370-2-729715; **Luxembourg** +352-496098; **Malta** Fax: +356-341087; **Morocco** Fax: +212-2-944040; **Malaysia** 60 (03) 755 5039; **Mexico** (5) 227 8967; **Netherlands** (036) 539 4911; **New Zealand** (09) 276 4157; **Nigeria** +234-1-521767; **Norway** (47) 23 373300; **Philippines** (632) 810 7246; **Poland** +48 (22) 22 66 84 305; **Portugal** (01) 4171717; **Republic of Ireland** 1 800 40 90 41; **Romania** +40-1-2123763; **Russia** (49) 621 759 8636 Fax: (49) 621 759 8611; **Saudi Arabia** +966-1-4010364; **Singapore** 0065 272 9200; **Slovenia** +386 61 1363528; **South Africa** (011) 886 2400; **South Korea** 02 569 6902; **Spain** (93) 201 4411; **Sweden** (08) 404 8800; **Switzerland** +41 (41) 799 6160; **Taiwan** (02) 736 7125; **Thailand** 66 (2) 274 07 08 (12 line); **Turkey** 0090 212 216 32 80; **United Arab Emirates** +971-4-694351; **United Kingdom** (0800) 521578; **USA** (800) 428 5433. **Venezuela** Fax: +0058-4810697; **Yugoslavia** +381 11 137163.



Roche Diagnostics GmbH  
 Roche Molecular Biochemicals  
 Sandhofer Strasse 116  
 D-68305 Mannheim  
 Germany