GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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European patent # 89 139 552
U.S. patent # 50 47 330

Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity assay kit #D05010.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated
by Bertin Pharma

Fabriqué en France
Made in France

#D11010
Version: 0117

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96 wells
Storage: -20°C
Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
Covered 96 well Microtiter plate	-	D08000.1 ea	1	-
G6PDH Reagent A	Red	D22010 1 ea	1	Solid
G6PDH Reagent B	Blue with white septum	D20010 1 ea	1	Solid
G6PDH Reagent C	Red with white septum	D21010 1 ea	1	Solid
10x G6PDH Reagent A Buffer	White with red septum	D25010 1 ea	1	Liquid
Sodium Hydroxide	Gold	D23010 1 ea	1	Solid
10x Dilution Buffer	Silver	D07000 1 ea	1	Liquid
Technical booklet	-	A11010	1	-
96 Well Cover Sheet	-	-	1	-

Each kit contains sufficient reagents for 96 wells. This allows for the assay of 24 samples in duplicate.

Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not mix different lot numbers
- Do not eat, drink or smoke in area in which kit reagents are handled
- Avoid splashing

Wearing gloves, laboratory coat and glasses is recommended when handling immunodiagnostics kit materials and samples.

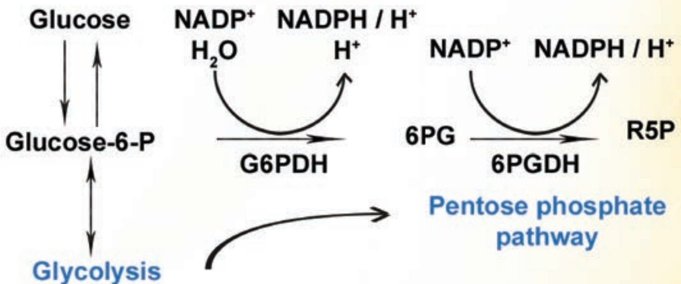
Sodium hydroxide solution is a potential harmful solution. When handling it, wear eye, hand, face and clothing protections to avoid any contact.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Background

The Glucose-6-Phosphate Dehydrogenase (G6PDH) is located into the cytosol of the cells.
First enzyme of the pentose phosphate pathway, G6PDH catalyzes the oxidation of glucose-6-phosphate into 6-phosphogluconate which is immediately converted into ribose-5-phosphate (R-5-P) by the 6-Phosphogluconate dehydrogenase(6PGDH).



The main function of the pentose phosphate pathway is the production of NADPH,H^+ and ribose-5-phosphate.
The NADPH,H^+ maintains all the species (antioxidant metabolite, glutathione) involved in oxidative stress regulation in reduced status to avoid damage from the reactive oxygen species (O^{2-}, H_2O_2 ...) ***[1]***.
The ribose-5-phosphate is the main sugar used for the nucleic acid synthesises ***[2]***.

Many diseases like neonatal jaundice, favism, haemolytic anaemia are linked to G6PDH deficiency ***[3]***. Overexpression seems to be potentially oncogenic ***[2]***.

Principle of the assay

The Glucose 6 Phosphate Dehydrogenase Activity assay kit provides a colorimetric method for detecting G6PDH activity which is specifically measured by following the appearance of NADPH,H^+ depending on the time period and protein concentration.

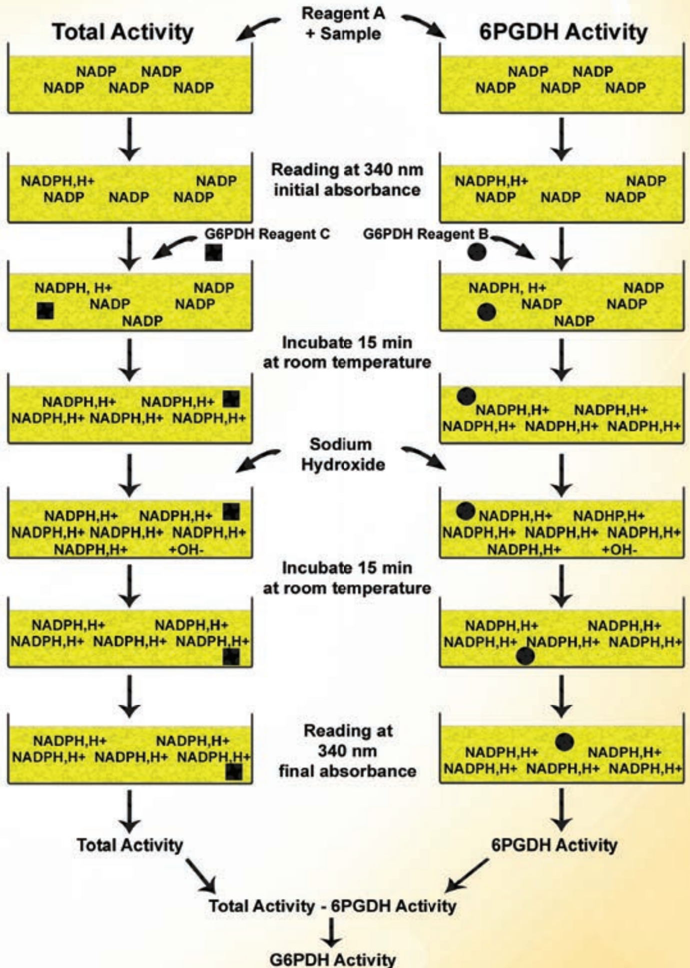
As mentioned previously, the NADPH,H+ from the pentose phosphate pathway is due to two enzymes: G6PDH and 6PGDH.

In order to measure specifically G6PDH activity, the assay is divided into two steps:

- First, Total Activity (G6PDH plus 6PGDH activity) is assessed by spectrophotometric measurement at 340 nm of NADPH, H^+ generation.
- Second, 6PGDH Activity is specifically evaluated by spectrophotometric measurement at 340 nm of NADPH,H^+ generation.

Finally, the G6PDH is obtained by subtraction of 6PGDH Activity from Total Activity.

The principle of the assay is summarized next page:


Reagent A
+ Sample
Total Activity
6PGDH Activity
NADP NADP
NADP NADP NADP
NADP NADP
NADP NADP NADP
Reading at 340 nm
initial absorbance
NADPH,H+ NADP
NADP NADP NADP
NADPH,H+ NADP
NADP NADP NADP
G6PDH Reagent C
G6PDH Reagent B
NADPH, H+ NADP
NADP NADP
NADP
NADPH, H+ NADP
NADP NADP
NADP
Incubate 15 min
at room temperature
NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+ NADPH,H+
Sodium
Hydroxide
NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+ NADPH,H+
NADPH,H+ +OH-
NADPH,H+ NADHP,H+
NADPH,H+ NADPH,H+ NADPH,H+
NADPH,H+ +OH-
Incubate 15 min
at room temperature
NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+ NADPH,H+
Reading at
340 nm
final absorbance
NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+
NADPH,H+ NADPH,H+ NADPH,H+
Total Activity
6PGDH Activity
Total Activity - 6PGDH Activity
G6PDH Activity

Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

- Precision micropipettes (10 to 1000 µL)
- Spectrophotometer plate reader (340 ± 10 nm filter)
- Microplate washer (or washbottles)
- Probe sonicator
- Multichannel pipette or repeating pipettor 100 µL and disposable tips
- Ultra pure water (item number #A07001.1L)
- Sodium chloride (NaCl) solution at 9 g/L
- Polypropylene tubes
- Total protein concentration method (BCA, Bradford)

Water used to prepare all EIA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Otherwise, organic contamination can significantly affect the enzymatic activity of the tracer Acetylcholinesterase (AChE).
Do not use distilled water, HPLC-grade water or sterile water.
UltraPure water may be purchased from Bertin Pharma : item #A07001.1L

Sample preparation

This assay has been validated to measure the G6PDH activity into lysates from cell cultures and erythrocytes.

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -80°C.

Total protein concentration in the sample must be measured in g/L (Bradford, BCA).
Dilution factors are suggested in these protocols.
However, the user must determine the optimal dilution factor.

Erythrocyte lysates

- Collect blood samples in tubes containing EDTA.
- Centrifuge at 3000 rpm. Remove the supernatant and keep the packed cells.
- Wash the cells with sodium chloride solution at 9 g/L and centrifuge at 3000 rpm during 5 minutes. The washing step must be repeated three (3) times.
- Add 1 mL of cold (+4C°) Ultra Pure water and centrifuge the tube at 3000 rpm during 5 minutes
- Measure the protein concentration in the sample.

In case of erythrocyte lysate, the recommended dilution with Dilution Buffer is 1/100.

Cell culture lysates

- Wash the cells with sodium chloride solution at 9 g/L and centrifuge at 3000 rpm during 10 minutes. The washing step must be repeated three (3) times.
- Add 1 mL of Dilution Buffer and lyse the cells by pulse sonication during 30 seconds with a probe sonicator.
- Measure the protein concentration in the sample.
- In case of cell cultures lysates, it is recommended to dilute the sample at 1 mg/mL with Dilution Buffer.

Reagent preparation

Unless specified, all reagents need to be brought to room temperature (around +20°C) prior to the assay.

Buffer A

Dilute 2 mL of 10x G6PDH Reagent A Buffer #D25010 with 18 mL of Ultra Pure water.
Stabilty at +4°C: 5 days

Dilution Buffer

Dilute 2 mL of 10x Dilution Buffer #D07000 with 18 mL of Ultra Pure water.
Stability at +4°C: 5 days

The remaining 10 x Dilution Buffer must be stored at -20°C until the next use.

Reagent A

Reconstitute the vial #D22010 with 10 mL of Buffer A. Mix thoroughly by gentle inversion.
Stability at +4°C: 5 days

Reagent B

Reconstitute the vial #D20010 with 3 mL of Ultra Pure water. Mix thoroughly by gentle inversion.
Stability at +4°C: 5 days

Reagent C

Reconstitute the vial #D21010with 3 mL of Ultra Pure water.
Mix thoroughly by gentle inversion.
Stability at +4°C: 5 days

Sodium Hydroxide

30 minutes before use, reconstitute the vial #D23010 with 5 mL of Ultra Pure water.
Stability at +4°C: 2 months

This step is exothermic, the water must be added carefully

Assay procedure

Unless specified, all samples and reagents must reach room temperature prior to performing the assay. Use different tips to pipet the different reagents.

Plate set-up

A plate set-up is suggested hereafter.
The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

TOTAL ACTIVITY (columns 1–6) | 6PGDH ACTIVITY (columns 7–12)

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S9	S9	S17	S17	S1	S1	S9	S9	S17	S17
B	S2	S2	S10	S10	S18	S18	S2	S2	S10	S10	S18	S18
C	S3	S3	S11	S11	S19	S19	S3	S3	S11	S11	S19	S19
D	S4	S4	S12	S12	S20	S20	S4	S4	S12	S12	S20	S20
E	S5	S5	S13	S13	S21	S21	S5	S5	S13	S13	S21	S21
F	S6	S6	S14	S14	S22	S22	S6	S6	S14	S14	S22	S22
G	S7	S7	S15	S15	S23	S23	S7	S7	S15	S15	S23	S23
H	S8	S8	S16	S16	S24	S24	S8	S8	S16	S16	S24	S24

S: Samples

We suggest that you record the content of each well on the template sheet as indicated above

We suggest that each sample is assayed at least in duplicate for the both Total Activity and 6PGDH Activity.

> Dispense 50 µL of diluted samples (see samples preparation section) into appropriate wells.

> Dispense 100 µL of Reagent A into each well.

> Read the **Initial absorbance** at 340 nm.

> Prepare the Sodium Hydroxide solution 30 minutes before its addition to wells.

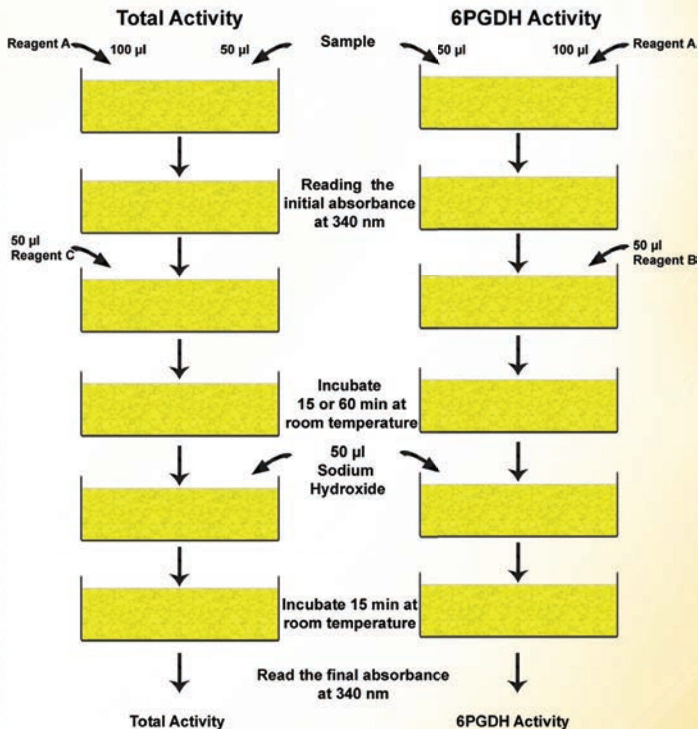
> Add 50 µL of Reagent B for 6PGDH Activity or Reagent C for Total Activity into the appropriate wells.

> Incubate at room temperature for exactly:
> - 15 minutes for cell culture lysates, or
> - 60 minutes for erythrocyte lysates.

> Stop the reaction with 50 µL of Sodium Hydroxide solution.

> Incubate 15 minutes at room temperature.

> Read the **Final absorbance** at 340 nm.


Total Activity
6PGDH Activity
Reagent A
100 µl
50 µl
Sample
50 µl
100 µl
Reagent A
Reading the initial absorbance at 340 nm
50 µl Reagent C
50 µl Reagent B
Incubate 15 or 60 min at room temperature
50 µl Sodium Hydroxide
Incubate 15 min at room temperature
Read the final absorbance at 340 nm
Total Activity
6PGDH Activity

Short Protocole (volumes are in µL)		
Volume / Wells	Total Activity	6PGDH Activity
Sample	50	
Reagent A	100	
Read the initial absorbance at 340 nm (*Inititial Abs*)		
Reagent B	-	50
Regent C	50	-
Incubate the plate 15 or 60 minutes at RT		
Sodium Hydroxide	50	
Incubate the plate 15 minutes at RT		
Read the final absorbance at 340 nm (*Final Abs*)		

Data analysis

The enzyme unit (U) is defined as the amount of enzyme that will catalyse the conversion of 1 µmol of substrate per minute.

Total Activity & 6PGDH Activity

For each sample, the **Total Activity** and **6PGDH Activity** must be determined as below:

- Determine the average initial absorbance (Initial Abs) of each sample duplicate for Total Activity wells and 6PGDH Activity wells.
- Determine the average final absorbance (Final Abs) of each sample duplicate for Total Activity wells and 6PGDH Activity wells.

- Calculation of ΔA (min^{-1}) for Total Activity

$$\Delta A_{total} = (\text{Final Abs}_{Total} - \text{Initial Abs}_{Total}) / \text{Incubation time (min)}$$

- Calculation of ΔA (min^{-1}) for 6PGDH Activity

$$\Delta A_{6PGDH} = (\text{Final Abs}_{6PGDH} - \text{Initial Abs}_{6PGDH}) / \text{Incubation time (min)}$$

- Calculation of Total Activity (U/g)

$$F(\mu mol/L) \times \Delta A_{Total}(min^{-1}) / [\text{protein (g/L)}]$$

F is a specific factor for this assay and is equal to $1{,}6.10^3$ µmol/L.

> Calculation of 6PGDH Activity (U/g)

$$F(\mu mol/L) \times \Delta A_{6PGDH} (min^{-1})/[protein\ (g/L)]$$

F is a specific factor for this assay and is equal to 1,6.10³ µmol/L.

G6PDH Activity

Total Activity (U/g) - 6PGDH Activity (U/g)

Calculation example for cells lysate

[Total Protein] = 0.99 g/L
Absorbance average at 340 nmm:

	6PGDH Activity		Total Activity	
	Initial Abs	Final Abs	Initial Abs	Final Abs
Sample	0.220	0.867	0.255	1.352

Incubation time: 15 minutes

> Calculation example of ΔA

For Total Activity:

$$\Delta A_{Total} = (Final\ Abs_{Total} - Initial\ Abs_{Total})/Incubation\ time$$

$$\Delta A_{Total} = (1.352-0.255)/15 = 73.10^{-3}\ min^{-1}$$

For 6PGDH Activity:

ΔA_{6PGDH} = (Final Abs_{6PGDH}-Initial Abs_{6PGDH})/Incubation time

ΔA_{6PGDH} = (0.867-0.221)/15 = 43.10^{-3} min^{-1}

> Calculation of Activity

Total Activity = (F x ΔA_{Total})/[Protein]
Total Activity = ($1,6.10^{3}$ x 73.10^{-3})/0.99 = 118 U/g

6PGDH Activity = (F x ΔA_{6PGDH})/[Protein]
6PGDH Activity = ($1,6.10^{3}$ x 43.10^{-3})/0.99 = 69 U/g

> Calculation of G6PDH Activity

G6PDH Activity = Total Activity - 6PGDH Activity
G6PDH Activity = 118-69 = 49 U/g

Assay validation and characteristics

G6PDH Activity Assay kit has been validated by using lysates from cell cultures.

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography ***[4, 5]***.

Precision

Inter-assay (n = 2)

Sample	G6PDH activity U/g	SD U/g	CV (%)
1	68.1	10.8	8.5
2	51.0	14.1	12.1

For inter-assay validation, the number of replicates (n) is equal to 2 each levels of QC. The 2 validation levels were measured in 5 independant runs.

Intra-assay (n = 10)

Sample	G6PDH activity U/g	SD U/g	CV (%)
1	41.8	5.3	12.8
2	87.0	11.3	13.1

For intra-assay validation, the number of replicates (n) is equal to 10 each levels of Qc. The 2 validation levels were measured in a unique run.

> Sensitivity (U/L)

The limit of detection is the G6PDH Activity, on a negative control (buffer without any protein) after 15 minutes incubation. During the validation process, the sensitivity was determined at 0,49 U/L

▷ Interference

The following reagents were tested for interference in the assay:

Buffers	Tris (55 nM)	No
	PBS (1x)	Yes
	HEPES (125 nM)	No
	Borate (100 nM)	No
Chelators	EGTA (1 nM)	No
Detergents	Triton X-100 (1%)	No
Solvents	Ethanol (5%)	No
	DMSO (5%)	No
Others	BSA (0.25%)	No
	SDS (1%)	Yes
	Glycerol (10%)	Yes

Troubleshooting

- **High dispersion of duplicates:** Poor pipetting technique, or one reagent has not been dispensed, or the assay was performed before reaching room temperature.
- **High dispersion of duplicates**:
  - poor pipetting technique,
  - one reagent has not been dispensed
  - the assay was performed before reaching room temperature...

These are a few examples of troubleshooting that may occur.

If you need further explanation, Bertin Pharma will be happy to assist you. Feel free to contact our technical support staff by phone (+33 (0)139 306 036), fax (+33 (0)139 306 299) or E-mail (bioreagent@bertinpharma.com), and be sure to indicate the batch number of the kit (see outside the box).

Bertin Pharma proposes EIA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Marketing Department by phone (+33 (0)139 306 260) or E-mail (marketing@bertinpharma.com).

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	TOTAL ACTIVITY						6PGDH ACTIVITY					
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Bertin Pharma, over the last decades, has been developing and marketing over 100 biomarker assays, pre-analytical products, kits, antibodies and biochemicals thanks to its innovative work in research and development.
Our core areas are orientated to inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, neurodegenerative diseases, HIV, prion diseases, pharmacokinetics and metabolism.

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