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ab102529 Glucose 6 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Glucose 6 Phosphate Dehydrogenase activity in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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Table of Contents

1. Overview 1
2. Protocol Summary 2
3. Precautions 3
4. Storage and Stability 3
5. Limitations 4
6. Materials Supplied 4
7. Materials Required, Not Supplied 5
8. Technical Hints 6
9. Reagent Preparation 7
10. Standard Preparation 8
11. Sample Preparation 9
12. Assay Procedure 11
13. Calculations 13
14. Typical Data 15
15. Quick Assay Procedure 19
16. Troubleshooting 20
17. Interferences 22
18. FAQs 22
19. Notes 23

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1. Overview

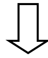
Glucose 6 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric) (ab102529) provides a simple method for detecting glucose 6 phosphate dehydrogenase (G6PD) activity in a variety of biological samples such as cell and tissue extracts, and biological fluids. The assay is based in the oxidation of glucose 6 phosphate (G6P) to gluconolactone by G6PD present in the sample. It is a simple, sensitive and rapid assay detects the activity of G6PDH in a variety of samples. In the assay, glucose-6-phosphate is oxidized with the generation of a product which is utilized to convert a nearly colorless probe to an intensely colored product with an absorbance at 450 nm. The color intensity is proportional to the G6PD activity present in the sample.

The assay can detect glucose 6 phosphate dehdyrogenase activity from as low as 0.04 mU G6PD in the well.

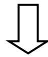
Glucose 6 Phosphate Dehydrogenase (G6PD, G6PDH, EC 1.1.1.49) catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone, the first and rate-limiting step in the pentose phosphate pathway (PPP). It is a critical metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The production of NADPH is of great importance for tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. The NADPH also maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage. Deficiencies in G6PD predispose individuals to non-immune hemolytic anemia.

2. Protocol Summary

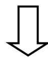
Standard curve preparation



Sample preparation



Add reaction mix



Incubate for 5 – 30 minutes at 37°C and measure absorbance (OD450 nm) in kinetic mode*

**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperatur e (after prep)
Assay Buffer V/G6PD Assay Buffer	25 mL	-20°C	-20°C
G6PD Substrate/G6PD Substrate (lyophilized)	1 vial	-20°C	-20°C
Developer Solution III/G6PD Developer (lyophilized)	1 vial	-20°C	-20°C
NADH Standard I/NADH Standard (0.5 µmol, lyophilized)	1 vial	-20°C	-20°C
Development Enzyme Mix IX/G6PD Positive Control (lyophilized)	1 vial	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm or fluorescence
- Double distilled water (ddH_2O)
- PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- (Optional) BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)
- (Optional) 10 kD Spin Column (ab93349) – to filter sample lysates if background noise is very high

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer V/G6PD Assay Buffer (50 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 G6PD Substrate/G6PD Substrate (lyophilized, 1 vial):

Reconstitute the G6PD substrate in 220 µL Assay Buffer. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C. Avoid freeze/thaw cycles. Once reconstituted, use within two months. Keep on ice while in use.

9.3 Developer Solution III/G6PD Developer (lyophilized, 1 vial):

Reconstitute Developer Solution III/G6PD developer in 220 µL ddH_2O. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C. Avoid freeze/thaw cycles. Once reconstituted, use within two months. Keep on ice while in use.

9.4 NADH Standard I/NADH Standard (lyophilized, 0.5 µmol):

Reconstitute NADH Standard I/NADH Standard in 400 µL ddH_2O to generate 1.25 mM (1.25 nmol/µL) NADH Standard solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at –20°C. Use within 2 months. Keep on ice while in use.

9.5 Development Enzyme Mix IX/G6PD Positive control (lyophilized, 1 vial):

Reconstitute positive control in 100 µL Assay Buffer. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C. Avoid freeze/thaw cycles. Once reconstituted, use within two months. Keep on ice while in use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Using 1.25 mM NADH standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	NADH 1.25 mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount NADH in well (nmol/well)
1	0	150	50	0
2	6	144	50	2.5
3	12	138	50	5.0
4	18	132	50	7.5
5	24	126	50	10
6	30	120	50	12.5

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

– We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
– We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation: 2 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 100 µL ice cold PBS (pH 6.5-8).
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Keep on ice for 10 minutes.
11.1.6 Centrifuge sample for 5 minutes at 4°C at 12,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new tube.
11.1.8 Keep on ice.
11.1.9 Optional: measure protein amount in the sample.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation ~10-100 mg).
11.2.2 Wash tissue with cold PBS.
11.2.3 Homogenize tissue in 100 µL of ice PBS (pH 6.5-8) with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
11.2.4 Centrifuge sample for 5 minutes at 4°C at 12,000 *xg* using a cold microcentrifuge to remove any insoluble material.
11.2.5 Collect supernatant and transfer to a new tube.
11.2.6 Keep on ice.
11.2.7 Optional: measure protein amount in the sample.

11.3 Plasma, serum and urine (other biological samples):

No sample preparation is required.

Δ **Note**: if sample background control wells show very high signal, you might need to remove interfering molecules with a 10 kD Spin Column (ab93349).

- Prewet the spin column with ddH_2O and spin down ddH_2O for 2 minutes at 10,000 x*g* at 4°C in a cold microcentrifuge. Remove ddH_2O from upper and bottom reservoirs.
- Add 100 µL sample lysate and spin down for 10 minutes at 10,000 x*g* at 4°C. Discard filtrate and collect upper fraction.

Δ **Note**: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- A positive control is provided to ensure the assay is working correctly. It shouldn't be used as standard or to extrapolate enzyme activity from the sample.
- If sample background control wells show high signal, we recommend performing an additional filtration step with our 10 kD Spin Column (ab93349) to remove interfering molecules. See Section 11 for more details/

Δ Note: Small molecules such as NADH present in cell or tissue extracts can generate background in this assay. We recommend that you set up Sample Background Controls to control for background noise.

12.1 Dilute Development Enzyme Mix IX/G6PD Positive control:

12.1.1 Dilute 5 µL Development Enzyme Mix IX/G6PD positive control (Step 9.5) in 495 µL Assay Buffer. This should be a suitable dilution to get 0.1-1 OD after 30 minutes incubation.

12.2 Reaction wells set up:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Sample Background Control wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Positive control wells = 10 µL diluted Development Enzyme Mix IX/G6PD positive control (adjust volume to 50 µL/well with Assay Buffer).

12.3 G6PD Reaction mix:

12.3.1 Prepare 50 µL of G6PD Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	46	48
G6PD Substrate	2	0
Developer Solution III/G6PD Developer	2	2

12.3.2 Add 50 µL of Reaction Mix into each standard, positive control and sample wells.
12.3.3 Add 50 µL of Background Reaction Mix into the background control sample wells.
12.3.4 Mix thoroughly.

12.4 Measurement:

12.4.1 Measure output at OD 450 nm on a microplate reader in kinetic mode for at least 5-30 minutes at 37°C protected from light.

Δ Note: Incubation time depends on the G6PD activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T1 and T2) to calculate the G6PD activity of the samples. For standard curve, do not subtract A2 from A1. Standard curve can also be read in end point mode (i.e. at the end of incubation time).

13. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
– Use only the linear rate for calculation.

13.1 Standard curve calculation:

13.1.1 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.1.2 Average the duplicate reading for each standard.

13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of G6PD activity in the sample:

13.2.1 For all reaction wells (including background control samples), choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding OD values at those points (OD1 and OD2)

13.2.2 Calculate ΔOD for sample as follows:

$$\Delta OD450nm = A2 - A1$$

13.2.3 Determine the background corrected change in colorimetric intensity for each well of sample by subtracting the ΔOD value of the background control (BC).

13.2.4 G6PD activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

$$G6PD\ Activity = \left(\frac{B}{\Delta T x\ V}\right) * D$$

Where:

B = amount of NADH in sample well calculated from standard curve (nmol).

ΔT = linear phase reaction time T2 – T1 (minutes).

V = original sample volume added into the reaction well (mL).

D = sample dilution factor.

G6PD activity can also be expressed as mU/µg of total protein in the sample.

Unit definition:

1 Unit G6PD activity = amount of G6PD that will generate 1.0 µmol of NADH per minute at 37°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

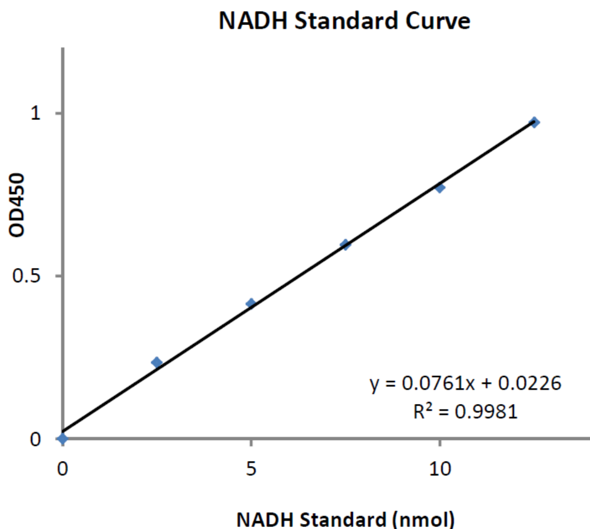


Figure 1. Typical NADH standard calibration curve.

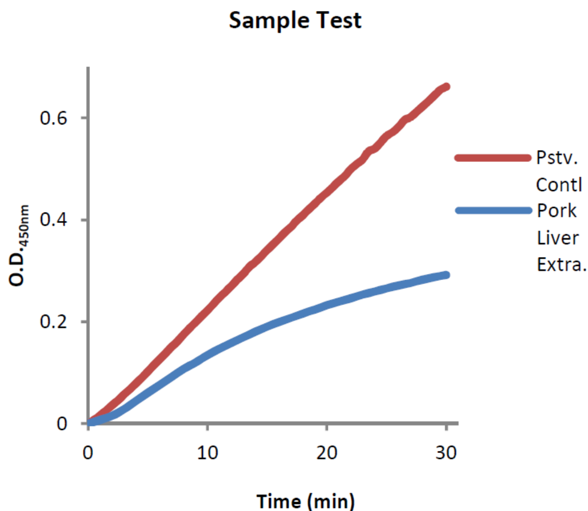


Figure 2. Kinetic curves showing G6PD activity in mouse liver extracts (blue) and positive control included in the kit (red).

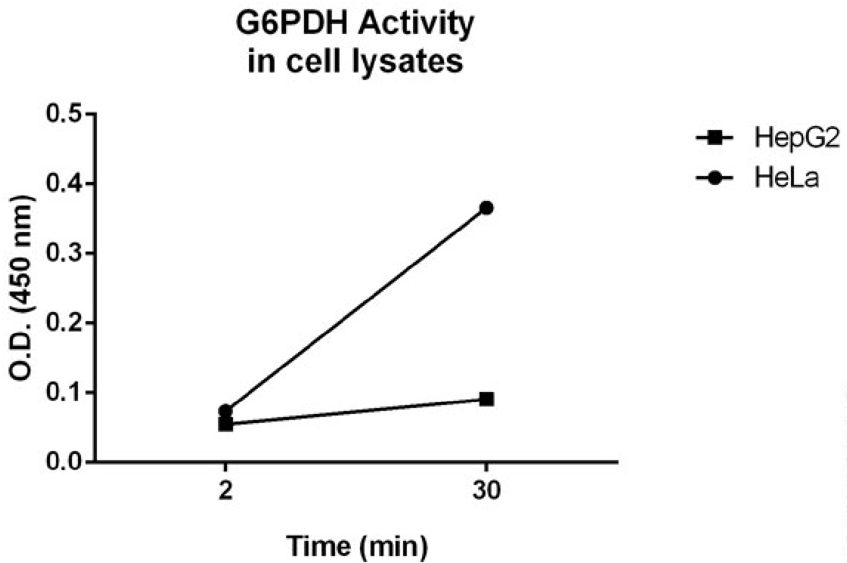


Figure 3. Kinetic curves showing G6PD activity in human cell lysates (4 x 10^6 cells/mL). Samples were undiluted.

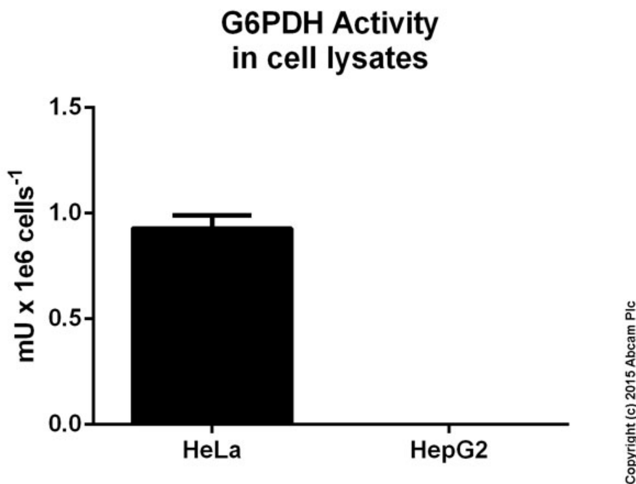


Figure 4. G6PD activity activity in human cell lysates (4 x 10^6 cells/mL). Samples were undiluted. Graph created from data obtained in Figure 3.

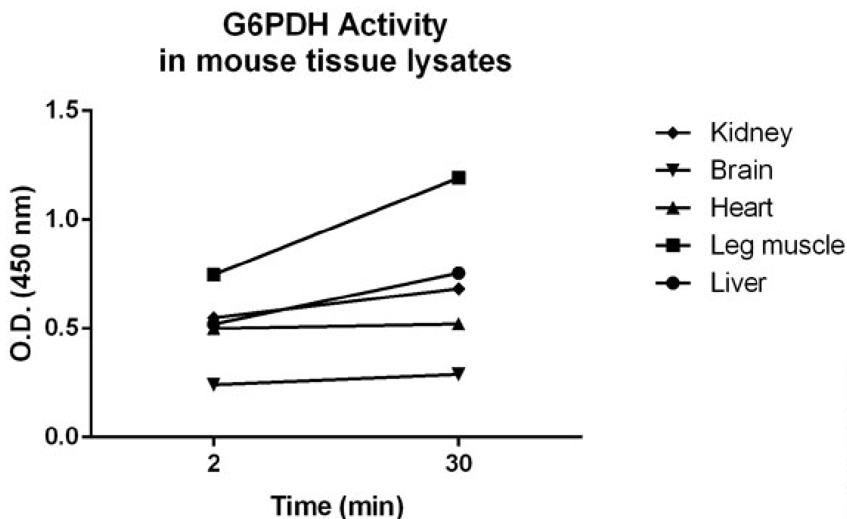


Figure 5. Kinetic curves showing G6PD activity in mouse tissue lysates. Samples were undiluted (protein concentration varied from 7-17 mg/mL).

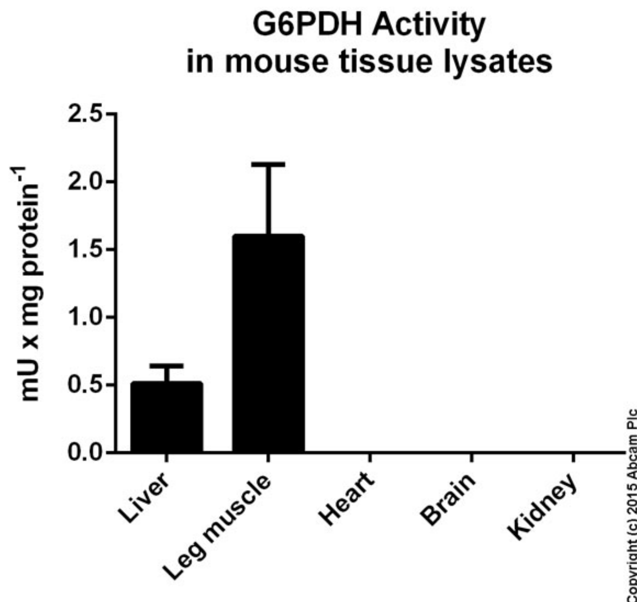


Figure 6. G6PD activity in mouse tissue lysates. Samples were undiluted (protein concentration varied from 7-17 mg/mL). Graph created from data obtained in Figure 5.

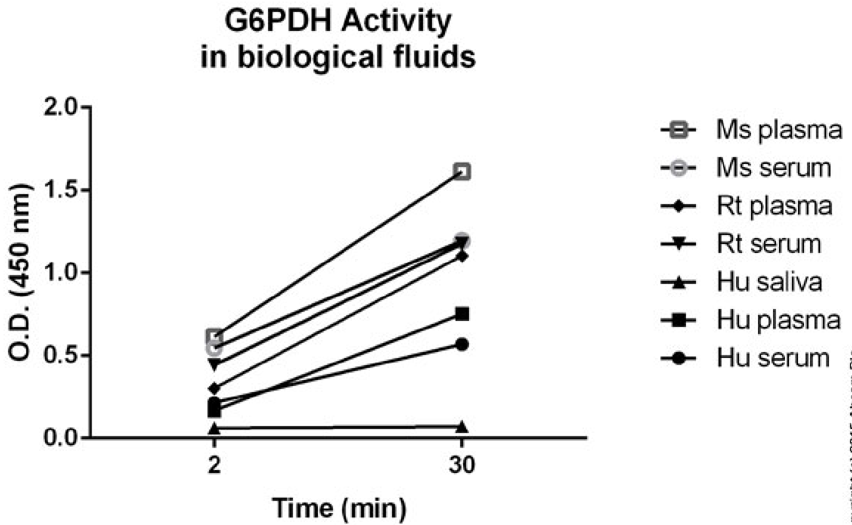


Figure 7. Kinetic curves showing G6PD activity in biological fluids. Samples were undiluted.

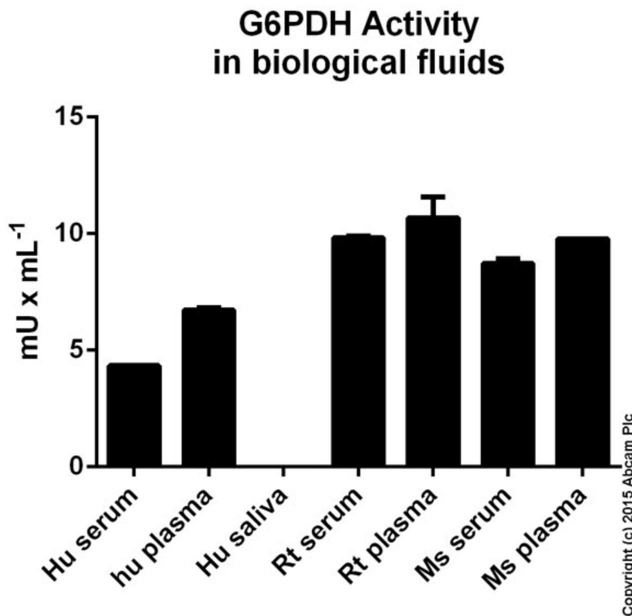


Figure 8. G6PD activity in biological fluids. Samples were undiluted. Graph created from data obtained in Figure 7.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare NADH standard dilution [2.5 – 12.5 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL), diluted (1:10) positive control and background sample control wells (50 µL).
- Prepare a master mix for G6PD Reaction Mix and (if appropriate) a master mix for Background Reaction Mix:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	46	48
G6PD Substrate	2	0
Developer Solution III/G6PD Developer	2	2

- Add 50 µL Reaction to standard, sample and positive control wells.
- Add 50 µL Background Reaction Mix to Sample Background control wells.
- Measure plate at OD 450 nm on a microplate reader in a kinetic mode at 37°C for 5-30 minutes protected from light.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

– NADH present in the sample can cause high background

18. FAQs

Q. What is the positive control?

A. The positive control is glucose 6 phosphate dehydrogenase from *Leuconostoc mesenteroides*, purified from *E. coli*.

Q. Why is NADH used in this assay instead of NADPH? Will NADPH interfere with the assay?

A. The first step of the reaction where G6PD converts G6P to the gluconolactone is a very specific reaction. The NADH formed in this reaction reacts with the probe to generate a colored product which is measured at OD 450 nm. Since the step is very specific, NADPH should not interfere with the assay.

Q. Can I use blood in this kit?

A. We have not tried this product in blood. Since blood is colored, it will interfere with the colored of the reaction and produce high background on the readings/ Also, since this is an enzymatic assay, you cannot use a filter to remove the proteins from blood to make it colorless; otherwise, you would lose all the enzymes as well, including the G6PD. We would recommend using plasma or serum.

19. Notes

Technical Support

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For all technical or commercial enquiries please go to:

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